δ^{13}CPLFA Analysis of the Microbial Community Composition within Peat Depth Profiles in Response to Deep Peat Warming and Environmental Conditions

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Dedication

This thesis is dedicated to:
My family for their undying support and unconditional love my entire life.
My friends who helped me keep going when I was discouraged.
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Abstract

Peatlands represent a critical and unique natural resource, especially in their role as carbon sinks. As much of the world's peatlands are located in Northern ecosystems where the climate is changing at a rapid pace, there is great interest and concern with how the changing climate will influence them. The microbial community is a crucial aspect of the peatland ecosystem in terms of mediating biogeochemical cycling. ^{13}CPLFA analysis was used to characterize the microbial community and provide isotopic information about microbial carbon use through peat depth profiles taken from the Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE) Project; an extensive study of the response of peatlands to climatic manipulation in the Marcell Experimental Forest in northern Minnesota. This analysis took place just before, and during, deep peat warming (at 2 meters) with temperature treatments (+0.0, +2.25, +4.5, +6.75, +9.0). Pre-warming sampling occurred in June 2014, with warming stabilizing around September 2014. These sampling dates were used to characterize the microbial community. The June 2015 sampling date was used to determine if the microbial community and carbon use responded to the deep peat warming. Strong vertical stratification indicates that depth is the biggest determining factor in natural peatlands on the microbial community composition and carbon use. There was also considerable natural variation of the microbial community over time, which will prove challenging in determining treatment effects. The surface of the peat profile had the most change over time, and had the strongest correlation with environmental variables, suggesting that the microbial community in the surface of the peat profile is more responsive to external conditions than deeper peat. The analysis of microbial communities before treatment initiation revealed that there were significant relationships of the microbial community with temperature, mostly related to depth under natural conditions. The deep peat warming enhanced that existing sensitivity. The fungal community in particular responded to the warming treatment, with increased relative abundance and use of newer carbon under higher temperatures. Decreased anaerobic bacterial relative abundance and actinomycete abundance in the top 20cm in response to warming indicates a microbial community shift towards fungal abundance, especially in the surface of the peat profile. In summary, fungal communities, and surface microbial communities in general could be the main drivers of change in peatlands under warming. This will have an especially big impact on nutrient cycling within the peatland ecosystem.

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CHAPTER ONE INTRODUCTION

Peatlands are a valuable natural resource, storing roughly one third of all global soil carbon (Joosten, 2009). Peatlands are found mostly in the northern hemisphere and form because their decomposition is restricted by environmental conditions such as low mean temperature and acidic, anoxic conditions within the soil (Dorreppaal et al, 2005). The plants that grow in peatlands also tend to be difficult to decompose (recalcitrant) (Dorreppaal et al, 2005). The source of hydrology determines whether a peatland is classified as a ombrotrophic bog (precipitation fed) or a minerotrophic fen (groundwater fed). The source of hydrology greatly impacts the diversity and pH of the peatland (Gorham, 1992). Bogs have a pH of around 4 with lower biodiversity and low nutrient contents. Fens have a pH of 7-8 and have higher biodiversity and higher nutrient contents (Gorham, 1992).

The accumulation of organic material in peatlands is vertically stratified, exhibiting layers of differing peat humification (Tfaily et al, 2014). The surface layer of peat is the acrotelm, which is less decomposed and supports plant growth such as emergent vegetation and woody trees and shrubs. The surface of the acrotelm is oxic and dynamic, containing the root/rhizome interface. The rest of the acrotelm fluctuates with the water table, being periodically oxic or anoxic (Tfaily et al, 2014). Peat found in the

middle of the profile, the mesotelm, has less decomposition than the deepest part of the peat profile, but more than the acrotelm, and is also periodically oxic as it is at the upper boundary of the water table (Boelter, Don H.; Verry, Elon S. 1977). The catotelm includes the deepest layer of a peat profile, consisting of very decomposed organic soil existing well below the water table above the mineral soil base. The deeper layers of the peat profile are anaerobic and below the penetration depth of living roots from vegetation on the surface (Sebestyen et al, 2011) (Figure 1).

Diverse species of plants exist across different peatland types. *Sphagnum* is a complex genus of peat moss that plays a large role in carbon storage in northern peatlands; especially bog ecosystems that are acidic and nutrient poor. Plant-microbe interactions are important in peatland ecosystems. *Sphagnum* acts as a plant host for a range of bacteria and fungi species, contributing carbon and oxygen to them (Medvedeff, 2015).

Microorganisms are present in enormous numbers in soil, and are drivers of biogeochemical cycling (Figure 2). In peatlands microbial activity presents as carbon cycling with CH4, CO2, and DOC fluxes (Limpens et al, 2008). The microbial structure and community of peatlands are driven by physical conditions within the peat such as pH, temperature, nutrient content/plant composition, moisture content, and oxygen availability. Understanding the microbial ecology of peatlands is crucial to understanding the carbon storage function of peatlands (Lin X et al, 2012).

Due to these physical conditions, the ecology and biogeochemistry of peatlands are complex, especially due to their seasonal and depth related variation. There are

hydrologic fluctuations over the year due to seasonal variation in temperature and precipitation regimes in northern peatlands. The specific climatic regimes at the sample site used in this study can be found in the methods section. Typically, northern peatlands have higher water tables and lower soil temperatures (in surface peat) in the spring, and lower water tables and higher soil temperatures (in surface peat) in the fall (Shi, X et al, 2015). The surface freezes over the winter with frost reaching limited depths, however deeper peat has less temperature fluctuation than surface peat (Sebestyen et al, 2011). The PLFA profiles of a Sphagnum dominated bog were significantly different at the beginning and end of the growing season (Anderson, 2010). Peat moss in the arctic showed fluctuation in the microbial community in response to freeze-thaw cycles, with temperature, oxygen, and DOC content and decomposition shifting as a result (Wynn-Williams, 1980). Seasonal fluctuation in microbial biomass and activity was significant in peatland soil (Potila, H, 2006). Changes in methane production, a result of microbial activity, shifted over time throughout the growing season in peat soils as a result of the interaction of temperature and organic matter quality (Bergman, 2000). PLFA analysis of a grassland over multiple years found the microbial responses to climate manipulation fluctuated over time (Gutknecht, 2012). Climate manipulation of a subarctic ecosystem found that changes to the microbial community composition and biomass took time to develop, with slight changes from year to year (Rinnan, 2007).

Peatlands are greatly involved in carbon cycling and storage. If the environmental conditions that lead to this storage are shifting, as is being observed due to climate change of warming in the northern hemisphere, peatlands may lose their ability to store

carbon (IPCC, 2013; Allison et al, 2011). This could turn peatlands from carbon sinks to a source of carbon through the release of carbon dioxide, becoming a positive feedback to climate change (Davidsson, 2006). A baseline understanding of peatland ecosystems and their biogeochemical cycling and how they vary with environmental conditions over time in order is needed in order to understand how these systems will react to climate change. Identifying this baseline and how it varies with environmental conditions, climate change, and peatland types is a gap in literature that needs to be filled (IPPC, 2007; Gorham et al, 1991).

Phospholipid Fatty Acid Analysis (PLFA) is an established method of evaluating the microbial community composition of water and soil. This method extracts lipids from peat which are then analyzed producing identifiable signature fatty acids which are indicative of specific microbial groups. These biomarkers are indicative of the microbial community. The community can then be evaluated. The biomass data can be used to determine the PLFA abundance which gives us an idea of where the community and to what extent along the depth profile.

Stable isotopes are valuable tools in evaluating biogeochemical cycling. The carbon cycle can be studied using the isotopes $^{12}C/^{13}C$. The $^{12}C/^{13}C$ ratio varies because of the fractionation of the isotopes when the carbon is being produced, respired, or assimilated (Boschker et al, 2002). ^{12}C isotopes are used preferentially to ^{13}C isotopes in biochemical reactions because they are lighter, leaving behind heavier ^{13}C isotopes and a less negative $^{12}C/^{13}C$ isotopic signature overall. The carbon that is assimilated by microbes in the ecosystem can be analyzed to find its isotopic signature. This is done by

extracting the lipids that make up microbial cell structures from bulk peat, which contain the carbon the microbes have metabolized. The ratio of $^{12}C/^{13}C$, or the isotopic signature of a material is expressed as $\delta^{13}C$. This value varies based on vegetative productivity and type as well as carbon burial (Rounick et al, 1986; Smith, 1979). The degree of isotopic fractionation by microbes can differ by microbial groups or by the entire microbial biomass as a whole. Newly photosynthesized carbon have a 13C depleted, i.e. more negative isotopic signature than older stored carbon deeper in the peat profile (O'Leary, 1988). This gives a vertical stratification effect of carbon age down the peat profile, giving an idea of how the carbon is being cycled in the peatland system as a whole. The Suess effect can observed as the addition of ^{13}C depleted CO_2 via anthropogenic addition (Esmeijer et al, 2012).

The aims of this project are to characterize the microbial community using bioindicators abundance and relative abundance, total PLFA abundance, and $\delta^{13}C$ trends along peat depth profiles from June and September 2014 and the ambient plots from June 2015. This chapter will investigate interannual variation, growing season variation, and variation in response to environmental conditions. This baseline information about microbial ecology in peatlands will be crucial in understanding the impact of changes like climate change. There is expected to be high depth impact on the microbial community. The microbial $\delta^{13}C$ is excepted to reflect the $\delta^{13}C$ of the bulk peat.

CHAPTER ONE METHODS

Experimental Site

This research was conducted as part of the Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE) project as sponsored by the Department of Energy and cooperatively operated by: the Oak Ridge National Laboratory, the U.S. Forest Service, and the Northern Research Station, among others. SPRUCE site is located in the S-1 Bog in the USDA Forest Service Marcel Experimental Forest in Grand Rapids, MN (Figure 3). The S-1 bog is a Black Spruce and Sphagnum dominated ombrotrophic bog with a perched water table (an impermeable layer of soil containing clay which prevents the water form draining) on the southern end of the boreal peatland forest that stretches from MN to Canada. Temperatures on site range from -45 to 38 degrees Celsius, with an average temperature of 18.9 in July and -15.1 in January. Average precipitation is 78cm with more occurring in the summer months than in winter, and snowfall is one third of the precipitation (Sebestyen et al, 2011).

The bog has roughly 30-100 cm of less decomposed surface peat which is porous and more drained, below which is well decomposed acidic peat with a pH of approximately 4. The anaerobic/aerobic zone fluctuates around 30cm. The peat profile has an average depth of 2.5m total before it reaches mineral soil. The maximum frost depth fluctuates between 0 and 50cm and is inversely related to snow depth on site. The bulk peat $\delta^{13}C$ ranges from -25 to -30, and increases with depth, indicating that lower in

the peat profile contains older stored carbon and surface depths containing newer, recently photosynthesized carbon (Hobbie et al., 2016).

The SPRUCE experimental design is described at greater length in chapter 2. Briefly, the design is climate manipulation with deep peat warming at multiple levels of warming.

Sampling Method

Bulk peat samples from three sampling dates were used for this analysis of microbial patterns over time. This analysis included the first two mass sampling dates in June and September of 2014, and the non-treatment plots from June 2015. At each sampling date two peat cores were sampled from 12 plots to a depth of 250cm (only to 200cm for June 2015). Two of the 12 plots (7 and 21) were unchambered controls and were only sampled to 100cm for all three sampling dates. These samples were bulked and homogenized in the field in increments of 10cm until a depth of 100cms and then in 25cm increments from 100cm to 250cm. The peat was frozen on dry ice immediately upon sampling and was then stored frozen at -80F until freeze dried. After freeze drying, the peat was ground under liquid nitrogen, weighed and combined evenly into 6 depth increments. In order to evaluate the depth profile with enough material for the PLFA analysis, sampled depth increments were combined as follows: 0-20cm, 20-50cm, 50-100cm, 100-150cm, 150-200cm, and 200-250cm.

These depth increments were based off of previous microbial research at this site indicating 30-50cm as a unique depth for the microbial community and biogeochemical

pathways, with microbial biomass trailing off after this point in the profile (Lin, X et al, 2014). This also correlates with bulk peat $\delta^{13}C$ data from this site with 50cm being where the $\delta^{13}C$ isotopic signature shifts significantly (Hobbie et al, 2016). The depth increments were also selected in accordance with this ecosystem's vertical stratification to find microbial community structural differences.

The top 20cm of the peat profile in this bog contains living senesced sphagnum. The first 30cm are transiently saturated, well drained, and have high hydraulic conductivities. The samples between 20-50cm contains decomposing sphagnum, with the 30-75cm depth zone classified as an intermediate zone with rapid decomposition due to water table fluctuations. The remainder of the deep peat profile contains well decomposed peat and is classified as the catotelm; which is permanently saturated, poorly drained, and has "low hydraulic conductivities". The water table is usually within 20cm of the surface and had a 40cm range of fluctuation (Wilson et al, 2016). The frost was reached 25cm in the year of 2012 (Lfaily, 2014; Sebestyen, Enclosure Hydrology SPRUCE All Hands Meeting). Soil carbon storage begins to increase after the first 20cm. The most soil carbon is located in the catotelm after the first 100cm.

PLFA Extractions

The amount of dry peat used for the PLFA extraction from each depth increment was dependent on both the limited amount of material and on microbial lipid abundance at each given depth increment, as determined by initial method testing (see appendix). Target weights were as follows: 2g dry weight for the 0-20cm depth increment, 6-8g dry

weight for increments 20-50cm, 50-100cm, and 100-150cm, and 10g dry weight for increments 150-200cm and 200-250cm.

The PLFA (Phospholipid Fatty Acid Analysis) method follows a modified Bligh and Dyer (1959) method modified both for δ^{13}CPLFA analysis (Herman et al 2011) and for extractions from peat (see appendix). The total lipid content was extracted from the peat using a .9:1:2 ratio of citrate buffer:$CHCl_3$:MeOH and then adjusted to a .9:1:1 ratio to allow for phase separation overnight. The $CHCl_3$ layer was evaporated and the total lipids were separated into their classes. The neutral, glycol, and phospholipid classes were fractionated using silica column chromatography with $CHCl_3$, acetone, and MeOH used to elute each lipid class. The phospholipid fraction was collected and used for the analysis because this fraction contains the microbial cell membranes. The phospholipids were methylated using methanol (adding a methyl ester group to each carbon to make them volatile and more easily read by the IRMS), to form Fatty Acid Methyl Esters. These FAMES were then transferred from their aqueous phase to their organic phase, and dissolved in hexane to be analyzed on a Gas Chromatograph Spectrometer. The 19:0 PC standard was used as a surrogate standard and the C 13:0 methylated FAME standard was used as an internal standard, and their addition was corrected for before analysis of the results proceeded.

Signature fatty acids (biomarkers) that indicate the microbial groups included here are: Gram positive bacteria (G+), Gram negative bacteria (G-), fungi (Fungi), acinomycetes (Actino), protozoa (P), and anaerobic bacteria (Anaerobic Bacteria). The naming of the FAMEs starts with the number of C atoms counted from the omega end

(ω), a colon, and the number of double bonds after the colon. The type of conformation (cis or trans), type of branching (iso or anteiso) of the FAMEs are given as the suffix c or t, and the prefix i or a. The suffix 10 ME is used to indicate the methyl group is attached at the 10^{th} C atom. The OH stands for hydroxyl and cyc stands for cyclopropane groups (Chowdhury, 2012). The specific lipids corresponding to these biomarkers in this project are as follows: fungi using 18:2 w6,9c and 18:1 w9c, gram negative bacteria using 18:1 w9t and 16:1 w7c, gram positive bacteria using 15:0 iso and 15:0 anteiso, acintomycetes using 16:0 10 me and 18:0 10me, protozoa using 20:4 w6,9,12,15, and anaerobic bacteria using 19:0 cyclo (Halbritter, 2002; Zelles, 1999; Balows, 1992; Thormann, 2006). $\delta^{13}C$ was averaged between lipids that indicated the same biomarker, and abundance and relative abundance were summed. Only microbial fatty acids (8:0-20:0) were included in the data analyses, therefore avoiding any eukaryotic lipids that could be from most plants (note exceptions regarding Sphagnum moss lipids).

Lipids indicative of microbes are found between 8:0 and 20:0. Eukaryotic and lipids found in vegetation are greater than 20:0. However the *Sphagnum* genus is unusual and lipids that make up these species end up falling in the microbial range. The lipid content of *Sphagnum* peat is mostly palmitic (16:0), linoleic (18:2), and linolenic (18:3) (Corrigan et al, 1976; Simola et al 1980; Dembitsky, 1993; Moore, 2015; Koskimies, 1980). Simola et al found that palmitic (16:0) and linoleic acids (18:2) were the most important of the phospholipids (1980). 16:0 was not included in the analysis of the microbial biomass and $\delta^{13}C$ data due to its high prevalence in sphagnum dominated

landscape especially in the top 20cm, as well as being not being a specific bioindicator. 18:2 and 18:3 were left in because they are also microbial bioindicators.

PLFA abundance of each fatty acid was converted from peak area to nmol g dry soil^{-1} using the 13:0 internal standard and adjusted for molecular weight per dry weight of peat. The PLFA abundance was calculated using the dry weight of peat after observing similar characterizations when comparing to the corrections for wet weight of the peat and the bulk density.

GC-IRMS Analysis and $\delta^{13}C$ calculations

The FAME extractions were analyzed using a coupled Isotope-ratio mass spectrometry and gas chromatography technique (IRMS-GC). An Agilent 7790A GC system and an Isoprime 100 IRMS system were utilized with a .32mm column, a flow rate of 2.1 mL/min, and a carrier gas of helium. This equipment detects the FAME's from the solution and measures the compound specific stable isotopic ratio ($\delta^{13}C$) between the heavier ^{13}C isotope and the lighter ^{12}C isotope. The results were calibrated against a 13C reference standard according to the following calculation (Team, 2005).

$$\delta^{13}\mathrm{C} = \left(\frac{\left(\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}\right)_{\mathrm{sample}}}{\left(\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}\right)_{\mathrm{standard}}} - 1 \right) * 1000\ {}^{o}/_{oo}$$

Equation 1. Equation to calculate the isotopic ratio of 12 to 13 carbon (Team, 2005).

The units for $\delta^{13}C$ are in per mil, where the standard is an established reference material.

Individual biomarkers were separated from a baseline and named by hand using individual chromatographs compared to the known reference standard (Boschker et al, 2002). Data clean-up included removing peaks less than 1nA in height because at less than this isotopic data can be unreliable. Lipids present at less than 0.5 mol% were not included to avoid noise.

Calculations for $\delta^{13}C$ included correcting for the additional carbon molecule added during the methylation of fatty acids to fatty acid methyl esters, by using the following formula:

$$\delta^{13}C_{PLFA} = [(C_{PLFA} + 1) \times \delta^{13}C_{FAME} - \delta^{13}C_{MeOH}]/C_{PLFA}$$

Equation 2. $\delta^{13}C$ correction formula for carbon added during methylation of the fatty acids during the PLFA procedure (Butler et al, 2003).

Standard outlier removal was utilized. The quartiles were calculated for the data to find the interquartile range (IQR), and the upper and lower bounds were found by adding and subtracting 1.5 times the IQR to the first and third quartile. The 8:0 peak was also left out of the $\delta^{13}C$ data because of inaccuracies at the beginning of IRMS-GC runs. The same sphagnum lipids were left out of the 13C and microbial biomass calculations. When more than one lipid was used for a bioindicator, the biomass and $\delta^{13}C$ values were averaged.

Peat abiotic parameters

Bulk density, gravimetric water content, and soil temperature calculations were made by others in the SPRUCE project and data was shared.

Gravimetric water content is the ratio of the mass of water per the dry mass of peat. Peat was weighed, dried, and weighed again with water content being grams H_20 (wet weight minus dry weight) divided by grams of dry peat (Hanson, P.J., 2015).

Bulk density is the measure of the peat sample dry mass divided by the peat sample volume. Bulk density measurements were taken in 2012 and these values were used for this analysis (Iversen, C.M., 2012).

Soil temperature was logged at depths of 0, 5, 10, 20, 30, 40, 50, 100, 200cm every 30 minutes. Temperature for this analysis was using the horizontal sensor position at Zone B (closest to where the peat core samples were taken) at the day of sampling (Hanson, P.J., 2015).

Statistical methods

The statistical program JMP was used for univariate analysis. Bivariate Oneway ANOVA analysis was used to determine significant linear relationships. Relationships were considered significant at $P < 0.05$. Plots were treated as random factors. Linear regression models were for the dependent variables of total microbial abundance, total mean $\delta^{13}C$, bioindicator abundance, relative abundance, and $\delta^{13}C$ of bioindicators. Independent variables included: sample date, month, and depth (nominal), % gravimetric

water content, measured soil temperature, and bulk density (continuous). The analyzed sample dates were June 2014, September 2014, and the ambient plots of June 2015, and the sample months were June and September. The main effects were considered to be depth, water content, bulk density, and time. The main effects were tested for, and effects were also analyzed separately within depth increments and sample dates, for example: Depth x Time, Temperature x Depth x Time, etc. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables.

Multivariate statistics are very useful for exploratory data analysis, and was used in this case to identify clusters of similarity in the lipid data. The statistical program R was used for NMDS ordinations of the lipid content for abundance, relative abundance, and $\delta^{13}C$. The distance matrix used was Bray.

CHAPTER ONE RESULTS

Gravimetric water content, bulk density, and temperature are all significantly autocorrelated to depth in a peatland ecosystem (Figure 4). Temperature decreases with depth, bulk density increases with depth, and gravimetric water content decreases from 0-100cm and then remains relatively constant for the remainder of the peat profile. At the surface of the peat profile the bulk density is very low compared to lower depths.

Seasonal variation in temperatures and water content at the SPRUCE site matches that seen in typical northern peatlands. There was greater water content in the surface of the peat profile in the June 2014 and June 2015 Ambient sampling dates than in September (Figure 5). September had higher soil temperatures than either June dates in ambient plots, reflecting natural variation instead of the treatment effect (Figure 6). Natural variation of these conditions can be seen with June 2015 ambient plots having much lower temperatures at the sampling date than June 2014 plots (Figure 6).

Microbial Abundance and Community Composition

Total Microbial Abundance

The major findings that impacted total microbial abundance were the variation based on location of depth in the peat profile, the variation over time, and the impact of other environmental conditions. Total microbial abundance decreased significantly with depth through the peat profile (Figure 7). Total microbial abundance was greatest in June 2014 at the 0-20 and 150-200cm depth increments and the lowest in the ambient plots of June 2015, demonstrating interannual variation between the June sampling dates (Figure 8). Total microbial abundance was positively related to temperature in June 2014 and in the entire year of 2014, most likely due to the autocorrelation with depth (Figures 9, 10). Total microbial abundance was positively related to water content and negatively related to bulk density, which were both strongly autocorrelated with depth (Figures 11,12).

However, total microbial abundance in the 0-20 cm depth increment was negatively related to water content.

Bioindicator Abundance

The major findings that impacted bioindicator abundance were the variation based on location of depth in the peat profile, the variation over time, and the impact of other environmental conditions. The abundance of all the bioindicators decreased significantly with depth through the peat profile (Figure 13, Table 1). P abundance was not present in the 0-20cm depth increment. There was clustering by depths for most depths in the NMDS for abundance (Figure 14, Table 4). The 0-20 and 200-250cm depth increments clustered closer together than the other depth increments. The 100-150 and 150-200cm depth increments also clustered more closely to each other. The 50-100cm depth increment was in the middle and on its own. The NMDS for sample date showed less clear separation (Figure 15, Table 4).

Interannual variation was demonstrated when June 2014 had significantly higher G+ abundance than any other sample date in the 0-20cm depth increment and the June 2015 Ambient plots had the lowest. Growing season variation was demonstrated when Anaerobic Bacteria abundance was significantly the greatest in June 2014 and the least in September 2014. In the 0-20 depth increment June 2014 had the highest Anaerobic Bacteria abundance and September 2014 had the lowest.

The abundance of all the bioindicators were positively related to temperature and water content, and negatively related to bulk density due to autocorrelation with depth (Table 2). However, within the 0-20cm depth increment F, G+, G-, A and AB abundance were all negatively related to temperature in June 2014. G-, G+, and AB abundance were positively related to temperature in the 50-100cm depth increment in June 2014, but not in September 2014. The G- and G+ abundance were negatively related to bulk density in the 200-250cm depth increment, and positively related to bulk density in the 0-20cm depth increment.

Bioindicator Relative Abundance

The major findings that impacted bioindicator relative abundance were the variation based on location of depth in the peat profile, the variation over time, and the impact of other environmental conditions.

The relative abundance of all the bioindicators significantly varied with depth except P (Figure 16). Fungi had the greatest relative abundance at the surface and then decreased until the 200-250cm depth increment. The relative abundance of G- also decreased down the peat profile until the 200-250cm depth increment. G+, Actino, P, and Anaerobic Bacteria relative abundance stayed relatively stable throughout the peat profile. In September of 2014 G+ relative abundance increased significantly with depth until the 200-250cm depth increment. The NMDS for relative abundance by depth found the 0-20cm and 200-250cm clustered separately and near each other (Figure 17, Table 4).

The 100-150 and 150-200cm depth increments clustered more closely to each other. The NMDS for relative abundance by sample date found that the June of 2015 ambient plots clustered more similarly then September and June of 2014, which could indicate interannual variation (Figure 18, Table 4).

Biomarker relative abundances demonstrated different patterns over time. Interannual variation was demonstrated when June 2015 Ambient plots had significantly higher G- abundance than any other sample date. Actino relative abundance was significantly greatest in June 2014 with June 2015 Ambient having the least. G- had the greatest relative abundance in the 20-50cm depth in the June 2015 Ambient plots and the least in June 2014. Growing season variation was demonstrated in the relative abundance findings as well. Actino relative abundance was significantly higher in June 2014 than in September 2014. Conversely G- bacteria had a higher relative abundance in September 2014 than June 2014.

Temperature, water content, and bulk density are all autocorrelated with depth, which significantly impacted all relative abundance. Fungal relative abundance was positively related to temperature, while Actino, Anaerobic Bacteria, and P were negatively related to temperature. Fungi, G-, and P were positively related to water content, while G+, Actino, and Anerobic Bacterial relative abundances were negatively related to water content. Fungal relative abundances were negatively related to bulk density, while G+, Actino, and Anerobic Bacteria were positively related to bulk density. Actino relative abundance was negatively related to temperature in general, with the strongest relationships in the 20-50cm depth increment in June 2014 and in the 0-20cm

depth increment in September 2014 (Table 2). In June 2014 G+ relative abundance was negatively related to temperature in general, with no depth having stronger or weaker relationships (Table 2). Fungal relative abundance was positively related to temperature in June 2014 and in the June 2015 Ambient plots, but no significant relationship was found in September 2014.

$\delta^{13}C$ Along Peat Depth Profile

Total $\delta^{13}C$

The major findings that impacted total $\delta^{13}C$ were the variation based on location of depth in the peat profile, the variation over time, and the impact of other environmental conditions. $\delta^{13}C$ significantly differed along the peat depth profile (Figure 19). $\delta^{13}C$ values significantly increased with increasing depth (as a continuous variable), reflecting the use of enriched (older) carbon is used at lower depths. $\delta^{13}C$ values are slightly lower at the very lowest depth increment. Growing season variation was demonstrated when June had significantly higher (older C) $\delta^{13}C$ values than in September (Figure 20). Total $\delta^{13}C$ values were highest in June 2014 (older C) than in September 2014 which had the lowest $\delta^{13}C$ values (newer C).

The $\delta^{13}C$ NMDS showed less clear separation by depth (Figure 21, Table 4) or sampling date (Figure 22, Table 4). Total $\delta^{13}C$ was negatively related to temperature and water content, which are both autocorrelated with depth (Figure 23). However, within specific depth increments, environmental conditions had significant impacts; the total

$\delta^{13}C$ was negatively related to temperature in the 50-100cm depth increment. In the 0-20cm depth increment in September 2014 total $\delta^{13}C$ was positively related to temperature.

Bioindicator $\delta^{13}C$ Signature

The major findings that impacted bioindicator $\delta^{13}C$ were the variation based on location of depth in the peat profile, the variation over time, and the impact of other environmental conditions. All bioindicator'$\delta^{13}C$ values except G+ changed significantly with depth (Figure 24). The bioindicator $\delta^{13}C$ profiles followed the same trend at the total $\delta^{13}C$. The microbes used older carbon lower in the peat profile and newer carbon at the surface of the peat profile. Growing season variation was demonstrated when the G- $\delta^{13}C$ was significantly more negative in September than in June, but the G+ $\delta^{13}C$ signature found the opposite to be true at the 200-250cm depth increment. In June 2014, Fungi and G- $\delta^{13}C$ signatures were negatively related to temperature in the 20-50 and 200-250cm depth increments respectively. In September 2014 G+ $\delta^{13}C$ is positively related to temperature in the 0-20cm depth increment.

CHAPTER ONE DISCUSSION

The goal of this analysis was to provide baseline information about the microbial community at an ombitrophic peat bog. The goal was also to provide information about

the change, or lack thereof, over time according to natural variation given the environmental conditions. Comparing June and September sampling dates could give information about the growing season's impact on the microbial community. Comparing June 2014 and the June 2015 ambient plots shows interannual fluctuation of the microbial community of the peatland from year to year. There is high variation across all data, showing how variable the microbial ecology of a peatland ecosystem is. The greatest variance in microbial abundance between plots was in the surface of the profile, as seen by the size of the error bars in Figure 5 and in the result that the 0-20 cm depth increment was more responsive to changes in abiotic factors than other depth increments (Tables 1-3). The 0-20cm and 20-50cm depth increment was also where the most changes occurring in the microbial community over time. This interaction between depth and time is important, $P<.0001$ using PERMANOVA analysis, especially considering the importance of depth on the microbial community. The surface may be a more sensitive and dynamic part of the peat profile to focus research on in the future.

While there was some change in microbial communities over time, depth was the strongest drivers of microbial variance. The vertical stratification effect of the peat profile strongly influenced the microbial community structure and abundance more than other factors, noting that temperature, soil moisture, and bulk density also strongly co-varied with depth and were related to microbial variance. All microbial bioindicator and total abundance decreased rapidly with increasing peat depth.

Bulk peat $\delta^{13}C$ from the SPRUCE site ranged from -25 to -30 along the peat depth profile (Hobbie, 2016). These values are slightly lower and more variable that what has

been found elsewhere 26.5 ± 2 ‰ (Broder, 2012). The isotopic signature could be impacted by sampling depth, vegetation type, and the type of hydrology present which impacts the rate of decomposition with microbial activity (Rounick et al, 1986; Smith, 1979). Microbial $\delta^{13}C$ values ranged from -26 to -33. The general $\delta^{13}C$ pattern reflects the signature of the bulk peat, with some microbial fractionation. The microbial isotopic signature is slightly more negative; containing a greater amount of 12C than 13C than the bulk peat due to the microbes preferential use of the 12C isotope and fractionation of the carbon isotopes.

Vertical stratification of microbial communities $\delta^{13}C$ Isotopic signatures

Vertical stratification was evident for microbial community structure, with bioindicator abundances and relative abundances significantly different with depth. Total microbial abundance and bioindicator abundance both decreased rapidly with depth due to the more stressful environmental conditions below the acrotelm. Fungi dominated the acrotelm, with actino becoming more prevalent beneath this surface layer of the peat profile. G+ and G- bacteria also became more abundant relatively below the acrotelm, and stayed more stable thorough the peat profile than fungi. Protozoa were absent in the acrotelm. Anaeorbic bacteria were present in low numbers throughout the entire profile. This stratification was expected, and is similar to results of other peatlands, although more wet peatlands such as ours seem more stratified than drier peatlands (Sundh, 1997).

The fungal bioindicator had the highest abundance but below the acrotelm it dropped to the level of the other microbial groups. Fungi decrease after the upper layer of a bog (Jaaiten, 2007). Fungi were missing at lower depths, but there were high numbers of ectomychorrizal fungi in the surface of the profile. 16S/18S analysis of the SPRUCE site showed that Fungi and Bacterial abundance declined with depth and were highly variable depending on the plot (Wilson et al, 2016; Kluber et al, unpublished, 2016). G+ and G- microbial groups decreased more slowly than fungi through the peat profile. The fungal biomarker decreases more quickly with depth than gram negative bacteria, and bacteria decreased more slowly down the profile in wet sites (Sundh, 1997). Bacteria are more adapted to the harsh environmental conditions in the acrotelm, especially the anaerobic bacteria.

The relative abundance of lipid bioindicators differed with depth. Bacterial and actino relative abundance decreased more slowly or even increased with depth than fungi in the lower acrotelm. The microbial community was impacted by the water table depth, with Gram negative and Gram positive bacteria and actino communities appearing the most at or just below the water table (Sundh, 1997). Conditions are well suited to bacteria in the uppermost anaerobic conditions where there is frequent fluctuation in aerobic and anaerobic conditions, and thus availability of diverse substrates (Thormann, 2006). The actino community sometimes peaks at intermediate depths and increases in upper dry bog regions (Sundh, 1997, Jaaiten, 2007). This could be due to fungi outcompeting the actino microbial group in the surface of the peat profile, causing them to have better success at the oxic bounds of the peat profile (Jaaiten, 2007).

The microbial community composition and structure interact to influence the carbon cycling and carbon storage present in an ecosystem (Herman et al, 2011). The soil fungal:bacterial ratio influences soil carbon storage, with higher fungal presence being linked to greater carbon storage capacity (Malik, 2016). Fungi dominate aerobic conditions and are major decomposers, perhaps even more significantly than bacteria. They also have the ability to translocate resources and grow quickly and extensively (Thormann, 2006). Understanding the microbial community composition leads to understanding about microbial use of soil carbon throughout the peat profile.

Across and within all dates total microbial $\delta^{13}C$ is significantly different by depth. Total $\delta^{13}C$ values were significantly negatively related to increasing depth. Less negative $\delta^{13}C$ lower in the peat profile indicates microbes at depth utilize older carbon or carbon more enriched in $\delta^{13}C$. The curve back towards a more positive $\delta^{13}C$ at the 100cm depth reflects the pattern seen in the bulk peat data as well. This depletion in $\delta^{13}C$ at depth is likely due to vegetative history at the site (Hobbie et al. 2016), while the depleted signal in surface peat is a result of new photosynthate and the Suess effect (Hobbie et al. 2016; Ehleringer et al, 2000). The microbes in the acrotelm are likely using newer surface carbon from plant exudates.

Bioindicator $\delta^{13}C$ values followed the same pattern as the bulk peat signature through the peat profile, with fractionation occurring (Šantrůčková, H, 2000). There was a difference in carbon use between the microbial groups. Different organisms have different levels of natural fluctuation, i.e. how strongly they select for 12C isotopes instead of 13C isotopes when incorporating carbon into their biomass. Of the microbial

community, fungi and actino groups break down older carbon whereas bacteria prefer to use newer labile carbon sources such as plant photosynthate. This is also reflected in the fractionation of the bioindicator carbon isotopes. Fungi, actino, and protozoa had higher $\delta^{13}C$ values; indicating the use of older carbon (Figure 24). Gram negative, positive, and anaerobic bacteria (especially gram negative bacteria) had lower $\delta^{13}C$ values; indicating the use of newer carbon, or a stronger fractionation of carbon as it is incorporated into microbial biomass.

Growing Season and Interannual variation in Microbial Communities and $\delta^{13}C$ signature

There were significant differences due to the growing season and the interannual variation on the microbial community composition as seen in the abundance and relative abundance of the bioindicators. This was identified by comparing the microbial results between the sampling dates; June 2014 to September 2014, and June 2014 to June 2015 Ambient plots. The natural variation across peatlands involves changes in plant growth and phenology, soil temperature, and soil moisture that impact the microbial community (Wynn-Williams, 1980; Bergman, 2000). One study found the PLFA of a peatland varied little over the growing season (Sundh, 1997). This was not corroborated by other microbial studies, which found that the differences in environmental conditions due to the growing season significantly impacted the microbial community structure and composition (Wynn-Williams, 1980; Potila, H, 2006; Bergman, 2000).

It is possible that patterns in the microbial community composition develop over the growing season. Temperature may have a bigger influence on the microbial community in June in the surface peat than in September due to the difficult growing conditions in the spring. Colder temperatures and thawing after the winter freeze in surface peat inhibit microbial growth and activity. The greater importance of temperature in the spring than in the fall can be observed with the microbial abundance and the majority of the relative abundance relationships with temperature occurring in June 2014 rather than in September of 2014. June 2015

There was interannual and growing season variance in the total $\delta^{13}C$ signature. The growing season variation could be due to microbes using newer plant photosynthate at the end of the growing season. The interannual variation is important to note because this needs to be taken into account when researching the long term effects of climate change.

Changes in the microbial community and carbon use over time displayed heterogeneity in depth responses. The top meter of surface peat had the most significant changes. Again, the surface being more responsive to change could be an important consideration for future peatland research.

Environmental Conditions and Relation to Microbial Community as a Predictive Mechanism

The relation of the microbial community to environmental conditions could help predict the response of the community to changes in those conditions. Climate change is

predicted to increase temperatures and decrease water content, especially at the peat surface. The relative abundance of fungi was positively related to temperature, but G+ was negatively related. Actino relative abundance was negatively related to temperature in the surface depth increments. Increased temperatures could lead to an increase in the fungal community and a decrease in the G+ and Actino community, especially at the surface. Total microbial biomass was positively related to temperature and negatively related to water content in the 0-20cm depth increment. Increased temperatures and drying at the surface could lead to increased total microbial abundance in the surface of the peat profile, possibly dominated by the fungal community.

The response of microbial carbon use to environmental conditions is especially important, although in the natural state of the peatland, the existing environmental conditions are due to their depth in the peat profile. However, within depth increments, the relationship of microbial carbon use to environmental conditions could be useful. The total $\delta^{13}C$ was negatively related to temperature in the 50-100cm depth increment. But in the 0-20cm depth increment in September 2014 the total $\delta^{13}C$ was positively related to temperature. This could suggest that increased temperatures in the fall leads to use of older carbon in the surface of the profile. But the increased temperature in the 50-100cm depth increment could lead to use of newer carbon by the entire microbial community.

The bioindicator $\delta^{13}C$ signatures also had relationships with temperature in the natural conditions of the peatland. In June 2014, the Fungal $\delta^{13}C$ signature was negatively related to temperature in the surface of the peat profile, suggesting that under higher temperatures the fungi community were using newer carbon, likely from plant

photosynthate. The increase in relative abundance of fungi with higher temperatures suggests that this growth is sustained by the use of newer carbon sources, possibly a result of increased mycorrhizal fungi. In September 2014 G+ $\delta^{13}C$ is positively related to temperature in the 0-20cm depth increment, suggesting that G+ bacteria used older carbon under higher temperatures in the surface of the peat profile, possibly being outcompeted for the newer carbon sources by fungi.

Caveats

June 2014 data may not be very useful (especially in the bioindicator $\delta^{13}C$ data) due to method development not being finalized. Less material was used in these earlier extractions than was needed as method development occurred. June 2015 non-treatment (ambient and control) plots that were used had the lowest biomass of all sampling dates, which may decrease the reliability of its data as well; especially $\delta^{13}C$ results which were found to be less reliable when corresponding to low biomass. Extremely low microbial biomass deeper in the profile led to difficulty in obtaining complete $\delta^{13}C$ and individual biomarkers data for the entire peat profile.

As discussed previously, there is a *Sphagnum* lipid contribution to the fungal biomarkers. They were kept in the analysis and could represent high fungal biomass in the surface peat but some could be from *Sphagnum* or *Sphagnum* endophytes.

There were additional caveats to note about the laboratory analysis, of which greater detail can be found in the Appendix. Laboratory method development was

required to adjust the PLFA extraction procedure from soil to the high organic peat material. There was a limited amount of material leading to an inability to re-run any of the samples. The IRMS-GC demonstrated temporal variance which led to difficulty in identifying lipid peaks.

CHAPTER ONE CONCLUSION

The June 2014, September 2014, and ambient plots of June 2015 were analyzed to gain a better understanding of the microbial community composition and carbon use under the natural conditions within the peat profile, and how it may vary over time. Vertical stratification had a huge effect on the microbial community composition. Environmental conditions are autocorrelated with depth under natural conditions. The 0-20 cm depth increment was more responsive to changes in abiotic factors than other depth increments. Natural variation in the microbial ecology was present to an extent. The plot and natural variation presents a challenge in determining future treatment effects. Depth related environmental variables and their relation to the microbial results gives an idea of how the community will respond in the change in climate, especially for the temperature increase and drying predicted. These relationships could be used as a predictive mechanism of the microbial community's response to changes in environmental conditions over time; such as fungi having a positive relationship with temperature whereas actinomycetes and Gram positive bacteria had a negative relationship. Microbial carbon use followed the same pattern as the bulk peat carbon signature with fractionation

occurring. Microbial groups fractionated the carbon differently. Overall, understanding the microbial community under natural conditions will be useful in interpreting the response to changes in environmental conditions over time.

Chapter 2: δ^{13}CPLFA Analysis of Microbial Community Composition along Peat Depth Profiles in Response to Deep Peat Warming

CHAPTER TWO INTRODUCTION

Arctic and boreal ecosystems may have a major role to play in earth's responses or feedbacks to climate change. Northern peatlands cover just 3% of the earth's land area but store about 30% of the total soil carbon (Blodau, 2002, Gorham 1991). The greatest warming from climate change is expected to take place in northern ecosystems where much of the world's peatlands exist (Davidson et al, 2006; Gorham 1991). Accelerated climate change also occurs at the edges of ecotones, such as the borders of the boreal forests where the SPRUCE study takes place (Rehm, E.M., 2015). A current research focus is on the yet unknown influence of this warming on the carbon cycling and carbon storage capacity of peatlands, especially peatlands in these sensitive areas with accelerated effects of climate change (with potential emissions mainly in the form of methane and carbon dioxide flux) (IPCC, 2007).

The microbial community structure and activity within peatland ecosystems are an intrinsic part of their biogeochemical cycling and ecosystem processes. Understanding the microbial community and composition of peatlands is crucial to understanding the bigger picture of peatland ecology and the possible response to climate change (Rinnan et al. 2007; Balser et al. 2005; Fraterrigo et al. 2005, 2006). Changes to ecosystems, such as what is occurring currently in northern peatlands, like climate change, increased CO_2,

nitrogen deposition, or natural or man-made disturbance, all influence microbial activity (Ajwa et al. 1999; Mayr et al. 1999). This change in microbial activity influences the biogeochemical cycling within the ecosystem such as decomposition, nitrogen mineralization, and carbon storage (Carreiro et al. 2000; Sinsabaugh et al. 2002; Henry et al. 2005; Sowerby et al. 2005).

Warming in northern latitudes increases soil respiration (Lin et al., 1999; Niinisto et al., 2004). Warming has led to increased microbial respiration in tundra soils (Hobbie 1996). However, there are other mixed findings regarding the influence of warming on soil respiration, with studies suggesting an increase, decrease, or no effect depending on the location and ecosystem (Juszczak, 2013). Response of microbial respiration to warming varied widely depending on differences in other environmental conditions on site; suggesting that the peat substrate, pH, and presence of microbial inhibitors have a bigger impact (Preston 2012). Warming has led to increased turnover rates of microbial abundance (Joergensen et al, 1990; Hagerty, 2014). Warming has also shown constant growth efficiency despite these increased microbial turnover rates (Hagerty, 2014).

Total microbial biomass has been found to increase initially under warming but decrease in the long term (Bardgett and Shine, 1999). The initial increase followed by a decrease could be due to microbial growth efficiency being altered under warming conditions, with the microbes shifting their use of carbon towards energy usage instead of biomass production (Zogg et al. 1997; Contin et al. 2000; Hyvonen et al. 2005; Schimel et al. 2007; Frey, 2008).

One of the two possible responses hypothesized for peatlands due to climate change is warming paired with drying. Warming at the surface of the peatland could lower water tables, increase oxygen, and increase decomposition (Wilson et al, 2016). Increased evapotranspiration due to warming would also lead to lower water tables, increased oxygen, and increased decomposition (Wilson et al, 2016). This is important because microbial respiration is limited by moisture, as well as temperature, and plant-microbe interactions are an important factor in microbial ecology. Plant root exudates feed microbial respiration, so when ecosystem GPP increases it correlates with increased microbial respiration (Yuste, 2007). Warming and drying of peatlands would lead to decreased methane production, but increased decomposition of old peat and biogenic CO_2 emissions to atmosphere (Fisk, 2003; Funk, 1994). The impact on carbon storage would be an increase in the mineralization (loss) of old stored carbon. However, the total release of CO_2 may be balanced by the decrease in methane emissions due to the drying and loss of methanogenic microbes (Zeng, X, 2016; White, 2008). The other possible response is warming without drying, thus increasing microbial activity and methane production, (biogenic emissions) (IPCC, 2013; Blodau, 2002; Limpens et al, 2008; Wilson et al, 2016). Methane is a significantly more potent greenhouse gas than CO_2, so this increase in emissions would be a concerning response of peatlands to warming.

Isotopic fractionation is a powerful tool in evaluating microbial carbon use. The microbe's preferential use of the lighter 12C isotope instead of 13C is expressed as a ratio as their isotopic signature $\delta^{13}C$ (Deniro, M.J., 1977). In a peatland, this leads to the more decomposed peat deeper in the peat profile containing carbon with a higher $\delta^{13}C$

signature and the less decomposed peat higher in the surface of the peat profile containing newer carbon with a lower $\delta^{13}C$ signature. Newer carbon from plant exudates that are metabolized by microbes also have a lower $\delta^{13}C$ signature indicating newer carbon. The microbes fractionate the $\delta^{13}C$ symbol found in the bulk peat as well as incorporating the carbon exudates from plant photosynthate (Abraham, W.R., 1998). The Suess effect can observed as the addition of 13C depleted CO_2 via anthropogenic addition (Esmeijer et al, 2012).

Microbes drive the humification (decomposition and mineralization) of stored carbon. Warming has led to the use of older carbon by the microbial community (McDonald 2005). Other studies found that warming shifted the microbial use of carbon toward both older and newer carbon sources, and the availability and source of carbon available to microbes differs based on soil temperature (MacDonald et al. 1995; Zogg et al. 1997; Andrews et al. 2000; Balser 2000). Warming has led to increased carbon mineralization in subsurface soils (Fierer, 2003). Subsurface peat carbon respiration increases with warming in the subarctic (Dorrepaal et al, 2009). Warming increases carbon decomposition rates as the quality of the carbon source decreases, such as the recalcitrant vegetative sources found in peatlands (Fierer, 2005). The response of microbial carbon use to warming shifts over time. An initial increase in the loss of soil carbon declines and stabilizes, which would indicate that microbial communities adapt to the warming by adjusting the amount of carbon that is allocated to growth (Allison et al, 2010).

Evaluating the response of microbes to climatic changes will aid in the evaluation of ecosystem vulnerability and function, as well as biogeochemical cycling of important nutrients (Balser et al, 2001). Microbe function is based on environmental conditions (pH, temperature, water and oxygen availability) and nutrient availability (Davidson, Janssens, 2006). Different parts of the microbial community serve different functions (and have different decomposition capabilities) and may respond differently to climate change (Whitaker, 2014). Fungal mycorrhizal communities especially have an important relationship with the plant and microbe community and their response to climate change will have ecosystem wide effects (Pendall et al. 2004). Actinomycetes, a type of filamentous bacteria that function similarly to fungi, break down especially recalcitrant material in the soil (Kuiters, 1990). Bacteria play an important role as well in soil decomposition, and can predominate in the deeper anaerobic layers of the peat profile (Mandic-Mulec, 2014). Gram negative bacteria prefer more oxic conditions and use of labile carbon, like fungi. Gram positive bacteria are more adaptable to anaerobic conditions and recalcitrant carbon sources (Bragazza, 2015). Methanogens and methanotrops play an important role in the cycling of carbon in peatland ecosystems, and in the emission rates of methane (Juottonen, 2012). Peatlands also contain sulfur oxidizing and reducing bacteria that play an important role in anaerobic mineralization of carbon (Pester, M, 2012). Protozoa prey on bacterial microbes and are an important part of soil ecosystems (Decamp, O, 1999).

Changes in the microbial carbon use can be investigated by their $\delta^{13}C$ signature under environmental changes such as warming, drying, or increased CO_2. Increased CO_2

in a grassland ecosystem caused microbes to shift their use from older soil organic carbon (SOC) to newer carbon from rhizodeposits because of increased plant root biomass (Cardon, Z. G, 2001). Microbial carbon use response to warming can be evaluated in this same way. Laboratory warming of boreal forest soil also found the initial microbial carbon use shifted to newer carbon sources (Dalias, P., 2001).

Warming has led to shifts in microbial community composition in the soil depending on the ecosystem (Zogg et al, 1997; Cregger et al, 2014; Peltoniemi, 2015). Warming the peat profile can influence the microbial community structure and composition by depth; explored by the shifts of the abundance of bioindicators and total PLFA abundance (Waldrop et al, 2006). The warming could also influence the microbial metabolism of carbon within the ecosystem (Dalias, P., 2001). This will provide information about how deep peat warming may influence organism and ecosystem vulnerability as well as changes in biogeochemical cycling of carbon. The microbial isotopic carbon signature can also be compared to the bulk peat signature to see if microbes are reflecting or fractionating the bulk peat carbon signature (Hobbie, 2016).

The aims of this analysis is to explore how deep peat warming may influence the microbial community. The effect of deep peat warming on the microbial community composition, PLFA abundance, and $\delta^{13}C$ cycling will be explored using the $\delta^{13}C$PLFA information from June 2015 analyzed, along the temperature gradient. With warming, if there is a shift towards a higher isotopic signal, it would mean the microbial use of carbon is shifting from newer carbon to older carbon.

CHAPTER TWO METHODS

See chapter one for the site description of the southern boreal peat bog used for this study, the sampling method, and the peat abiotic parameter collection methods.

The SPRUCE project is an extensive study of climatic manipulation of peatland. The warming treatments range evenly from ambient to +9 Degrees Celsius (Ambient, +0, +2.25, +4.5, +6.75, +9) with two repetitions of each treatment (Figure 25). Open topped chambers contain 3 meter long heating rods with treatments designed to reach target temperatures at 1.5 to 2 meters, radiating the heat upward through the rest of the peat profile. The treatments are the most clearly separated deeper in the peat profile whereas treatments at the surface of the profile are present but less distinct (Wilson et al 2016). The deep peat warming began after initial sampling in June 2014 and stabilized within 0.5 degrees Celsius of the target temperature by the second mass sampling date in September of 2014 (Wilson et al, 2016). The last mass sampling date for this thesis was June of 2015.

The June 2015 plots were used to investigate the effects of deep peat warming on the microbial community structure and carbon metabolism. The same field methods were used in the collection of this peat as described in the first chapter. The same depth increments were used for this analysis except the June 2015 sampling date were only sampled to a depth of 200cm rather than 250cm. These increments were 0-20cm, 20-50cm, 50-100cm, 100-150cm, and 150-200cm.

The same PLFA lab procedure, IRMS-GC procedure, and $\delta^{13}C$ calculations were used as described in the first chapter. Briefly, the lipids corresponding to the biomarkers used in this project are as follows: fungi using 18:2 w6,9c and 18:1 w9c, gram negative bacteria using 18:1 w9t and 16:1 w7c, gram positive bacteria using 15:0 iso and 15:0 anteiso, acintomycetes using 16:0 10 me and 18:0 10me, protozoa using 20:4 w6,9,12,15, and anaerobic bacteria using 19:0 cyclo (Halbritter, 2002; Zelles, 1999; Balows, 1992; Thormann, 2006).

Statistical methods

The statistical program JMP was used for univariate analysis. Bivariate Oneway ANOVA analysis was used to determine significant linear relationships. Relationships were considered significant at $P < 0.05$. Plots were treated as random factors. Linear regression models were for the dependent variables of total microbial abundance, total mean $\delta^{13}C$, bioindicator abundance, relative abundance, and $\delta^{13}C$ of bioindicators. Independent variables included: sample date, month, and depth (nominal), % gravimetric water content, measured soil temperature, and bulk density (continuous). The analyzed sample date was June 2015. The main effects were considered to be depth, water content, bulk density, and time. The main effects were tested for, and effects were also analyzed separately within depth increments and sample dates, for example: Depth x Time, Temperature x Depth x Time, etc. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables.

Multivariate statistics are very useful for exploratory data analysis, and was used in this case to identify clusters of similarity in the lipid data. The statistical program R was used for NMDS ordinations of the lipid content for June 2015 abundance, June 2015 relative abundance, and June 2015 $\delta^{13}C$. The distance matrix used was Bray. Due to a lower overall abundance, the June 2015 profile was analyzed to a depth of 100cm in the NMDS ordinations.

CHAPTER TWO RESULTS

As discussed in Chapter One, Variation in the microbial community is highly correlated with depth, as are other environmental. Gravimetric water content and bulk density are both significantly autocorrelated to to depth in a peatland ecosystem. Bulk density increases with depth, and gravimetric water content decreases after the surface and then remains relatively constant for the remainder of the peat profile. Due to the deep peat warming, the temperature profiles in June 2015 are very different than their natural state in chapter one (Figure 26).

Treated plots in June 2015 had slightly lower water contents in the surface meter of the peat profile than ambient plots, however the difference was not significant yet. This could be indicative of the start of warming and drying with more oxic conditions in the surface of the peat profile (Figure 27). The June 2015 sampling date occurred during a cold and wet spring, with some plots still frozen.

Microbial Abundance and Community Composition

Total Microbial Abundance

Total microbial abundance had an important relationship to temperature as well as other environmental conditions including depth. Total microbial abundance was positively related to temperature across all sample dates (Figure 28) this relationship was not significant in June 2015 (Figure 29). This could be due to depth having a stronger impact on total microbial growth than temperature at this beginning stage in the warming treatment, as seen by the negative relationship of microbial growth with depth in June 2015 (Table 5). Total microbial abundance was positively related to water content and negatively related to bulk density (Table 5).

Bioindicator Abundance

Bioindicator abundance had an important relationship to temperature as well as other environmental conditions including depth. In June 2015, Actino abundance was negatively related to temperature in the 0-20cm depth increment, (Figure 30), but when considered across all depth increments there was no relationship of Actino abundance with temperature (Table 5).

Fungi and G+ abundance significantly decreased with depth (Table 5). Fungi and G+ abundance were positively related to water content and negatively related to bulk density (Table 5). The NMDS for June 2015 indicated the microbial abundance was distinct according to depth, with each depth increment clustering separately (Figure 31,

Table 8). The NMDS that is colored and shaped by plot and treatment showed no real pattern of clustering (Figure 32, Table 8).

Bioindicator Relative Abundance

Bioindicator relative abundance had an important relationship to temperature as well as other environmental conditions including depth. Anaerobic bacteria relative abundance was negatively related to temperature only in the 0-20cm depth increment in June 2015 (Figure 33). Fungi relative abundance was positively related to temperature in June 2015 through the entire peat profile (Figure 34).

Fungi significantly decreased and Actino significantly increased with peat depth, although due to low overall abundance these indicators were only found to the depth of 50cm. Fungal relative abundance was positively related to water content and negatively related to bulk density. The relative abundance of G+, Actino, and Anaerobic Bacteria were all positively related to bulk density but not directly related to depth even though depth and bulk density are autocorrelated. (Table 6). The NMDS for June 2015 indicated the relative abundance was distinct according to depth, with each depth increment clustering separately (Figure 35, Table 8). The NMDS that is colored and shaped by plot according to treatment showed no real pattern of clustering (Figure 36, Table 8).

$\delta^{13}C$ Along Peat Depth Profile

Total $\delta^{13}C$

Total $\delta^{13}C$ had an important relationship to temperature as well as other environmental conditions including depth. The total $\delta^{13}C$ was negatively related to temperature when analyzed across all sampling dates, and within the 50-100cm depth increment (Figure 37). There was no significant relationship to temperature however when only considering the June 2015 data (Figure 38).

In June 2015, total $\delta^{13}C$ significantly increased with peat depth (Table 7). The 20-50cm depth increments had the lowest $\delta^{13}C$ values and the 50-200cm range had the highest values. In June 2015 total $\delta^{13}C$ was negatively related to water content and positively related to bulk density (Table 7).

The NMDS for June 2015 indicated there was no pattern of clustering for $\delta^{13}C$ by depth (Figure 39, Table 8). The NMDS that is colored and shaped by plot and treatment showed no real pattern of clustering (Figure 40, Table 8).

Bioindicator $\delta^{13}C$

The bioindicator $\delta^{13}C$ values were less useful due to June 2015 having the lowest microbial abundance of all of the dates, leading to an incomplete profile. There were no significant $\delta^{13}C$ findings for the Actino, G-, G+, and Anaerobic Bacteria biomarkers, and there was insufficient data for any findings for the P bioindicator. One bioindicator $\delta^{13}C$ had an important relationship to temperature. In June 2015 the Fungi $\delta^{13}C$ was negatively related to temperature but not significantly related to depth (Figure 41, Table 7).

CHAPTER TWO DISCUSSION

Microbial Community Composition Response to Deep Peat Warming

This analysis found significant responses of the microbial community to warming, but only certain parts of the community and often only in certain depth increments. The analysis of microbial communities before treatment initiation discovered that there were significant relationships of the microbial community with temperature, mostly related to depth, as discussed in Chapter one, so warming could have enhanced that existing sensitivity.

Actino abundance was negatively related to temperature in the 0-20cm depth increment in June 2015 which could mean the warming will decrease the abundance of this microbial group in the surface of the peat profile. Fungal and actino microbes were found to decrease at higher temperatures (Waldrop and Firestone, 2004). However, in non-peatland soil, long term warming resulted in decreased fungal biomarkers and increased bacterial biomarkers (gram positive and acinomycetes) (Frey, 2008). This variation in response is why ecosystem specific microbial research is critical.

The relative abundance of fungi was positively related to temperature in June 2015. This could mean that warming will lead to more growth of fungi relative to other organisms, especially in the surface peat. This shift in the microbial community composition to fungal dominated rather than bacteria dominated is most likely due to the increasing oxic conditions (Bragazza, 2015). The warming and drying of the actrotelm is preferential for fungal growth. Warming in fens led to increased fungal presence in the

surface of the peat profile but decreased bacterial abundance (Peltoniemi, 2015). This would impact the nutrient cycling of the peatland, as fungi play a large role in this ecosystem function. Ectomychorrizal (EM) fungi form a hyphal network with the plant community's root systems, providing water and nutrients to the plants and cycling carbon. EM fungi have been found to be impacted both positively and negatively by warming (van der Heijde, 2013). Warming was also found to either decrease or increase the abundance of arbuscular mycorrhizal fungi (Pendall et al, 2004). Sapotrophic fungi primarily break down non-living carbon and their response to warming has implications for carbon storage. Warming could increase the fruiting season of sapotrophic fungi while having less of an impact on EM fungi (Gange, E.C.). The SPRUCE peatland site has large numbers of ectomychorrizal fungi in the surface (Wilson et al, 2016). In boreal ecosystems, saprotrophic and EM fungi have competitive interactions, and mycorrhizal fungi can potentially slow decomposition rates of litter by hindering the faster decomposition rates of saprotrophic fungi (Bödeker, I, 2016).

The relative abundance of anaerobic bacteria was negatively related to temperature in the surface of the profile, which could mean the warming will decrease the relative abundance of anaerobic bacteria in the community at this depth. This could also be due to the increasing oxic conditions in the acrotelm, causing the anaerobic bacteria to be outcompeted by the fungal microbes.

Microbial $\delta^{13}C$ Response to Deep Peat Warming

In June 2015 the fungi $\delta^{13}C$ isotopic signature was negatively related to temperature. This was not the case in any other sampling dates, except in the 20-50cm depth increment in June 2014 when fungi $\delta^{13}C$ was also negatively related to temperature. This could indicate an existing sensitivity of fungal carbon use to temperature that the warming treatment enhanced, and that the fungal community is using newer photosynthate carbon as a result of the warming. If the increase in fungal relative abundance resulted from ectomycorrhizal fungi, then the warming is increasing ectomychorrihzal plant root biomass and colonization resulting in the fungi community using newer carbon from this network (Hobbie, 1999). If the increase in fungal relative abundance resulted from the growth of saprotrophic fungi, then this community is using newer carbon by taking up more root exudate. Saprotrophic fungi have a higher $\delta^{13}C$ than ectomychorrihzal fungi, tending to decompose older peat from dead or dying plant matter, however they could still be using the newer carbon root exudates under these warming conditions (Hobbie, 1999).

The SPRUCE bog also had an increase in microbial methane emissions from the 20-30cm depth increment with a more negative isotopic signature. This was not reflected in a $\delta^{13}C$ signature change in the bulk peat, indicating that newer photosynthate was being used by the microbial community rather than older carbon being decomposed (Wilson et al, 2016). Wilson et al. hypothesize that deep peat is recalcitrant to heterotrophic microbial decomposition, and so warming alone is not likely to impact carbon storage in the anoxic catotelm (2016). However, this may happen with longer

term warming, additional climate manipulation, and the fungal community response over time.

Fungi and bacteria ratios impact carbon storage ability of soil ecosystems (Malik, 2016). Increased fungal compared to bacteria ratio leads to higher carbon levels in the microbial biomass which could be related to fungal carbon needs being higher than bacteria carbon needs or fungal carbon use efficiency (CUE) (Bragazza, 2015). The microbial CUE changes under warming conditions depending on the microbial community physiology (Allison et al, 2010). Warming has been found to initially decrease carbon use efficiency, which can balance out over time if the microbial community adapts to the warming (Allison et al, 2010; Schindlbacher, 2011). Even though fungal abundance hasn't increased significantly yet in response to warming (although fungi relative abundance has), the fungal carbon use efficiency could have responded to the warming treatment, resulting in increased labile carbon use by the fungal community.

Warming in boreal ecosystems has shown that the function of fungi can be altered. Warming shifts the fungal community, leading to the increased ability of the microbial community to breakdown old carbon (Tresder, 2016). Essentially, the use of new carbon primes the system for loss of old carbon, after the labile carbon source is exhausted (Bragazza, 2015). Laboratory warming of boreal forest soil also found the initial microbial response was to exhaust the newer, labile carbon sources with a potential long term response of switching to older SOC (Dalias, P., 2001). Warming and an increase in the fungal community presence led to an increase of a carbon degrading

enzyme in the soil, resulting from the increase in labile carbon from vascular plants (Bragazza, 2013).

The analysis in chapter one found the fungal and actino $\delta^{13}C$ isotopic signature contained the oldest carbon of the microbial community. These microbes are both known for decomposing especially recalcitrant carbon (Kuiters, 1990; Pendall et al, 2004). So once the labile carbon sources are exhausted the fungi community could turn to the use of older carbon.

This analysis indicates that warming of the peatland increases the relative abundance of the fungal community, leading to an initial increase in the use of new carbon which could be followed by the use of old carbon. Longer term warming could lead to loss of soil carbon storage from the peatland, although shorter term indicates the microbial community is using the newer carbon sources that are produced because of the warming treatment.

General Responses to Temperature

Significant differences of the δ^{13}CPLFA between sample dates could be a result of a warming treatment response or natural variation, as the analysis in chapter one explored. June 2015 was a significant sample date for some microbial community composition findings.

June 2015 had the lowest total microbial abundance in the 0-20 and 150-200cm depth increments. This finding could suggest a warming response of decreased microbial abundance, however this would be unusual compared to other warming studies. The low

microbial abundance can be explained by the sampling date occurring during a cold and wet spring, with some plots in the sampling area still frozen over. Typically, warming and drying in peatlands increases vascular plant biomass which usually leads to increased microbial biomass (Bragazza, 2015). Total microbial biomass has been found to increase initially under warming but decrease in the long term (Bardgett and Shine, 1999). An initial increase in total microbial biomass under warming in a temperate grassland was hypothesized to be due to fast-growing bacteria and that the slower growing fungi and actinomycetes microbial groups were unaffected (Bardgett and Shine, 1999). However, fungi and actino microbial groups had significant responses to the warming in this study.

June 2015 had the lowest actino relative abundance of the June sampling dates which could indicate a warming response of a decrease in the actino relative abundance. This decline would concur with the negative relationship of actino abundance to temperature found in June 2015, and the positive relationship of fungi relative abundance.

Other parts of the bacterial community had significant findings by sample date. June 2015 had the least abundance of G+ bacteria in the 0-20cm depth increment. This could mean the warming decreased the G+ abundance in the surface of the peat profile in favor of fungi.

The G- and G+ communities in a peatland study increased with warming, which was found to be partially true in this analysis (Zogg, 1997). G- had the greatest relative abundance in June 2015, which could indicate a warming response of increased relative abundance of G- microbes in the 20-50cm and 50-100cm depth increments. This could be due to their preference for more oxic conditions and labile carbon sources.

Future SPRUCE Climatic Manipulation and Relationship to Initial Deep Peat Warming

Although this analysis only involved deep peat warming, the entire SPRUCE project investigates other climatic manipulation reflective of the predicted responses to climate change in the northern hemisphere (IPPC, 2007). In the case of the SPRUCE project, this involves atmospheric warming and CO_2 addition. This is important due to the fact that climate change involves changes in multiple factors that interact to impact ecosystems. The long-term nature of the SPRUCE study is also important due to the shifting responses of microbial ecology over time.

Additionally, the small response of microbial carbon use to the deep peat warming so far may be misleading. Many complicated feedback mechanisms may mask the temperature sensitivity of soils due to other shifting environmental conditions with climate change (Janssens and Davidsson, 2006). Changes in microbial carbon use could also take more time to develop.

Warming with increased CO_2 together can increase (Niinisto et al., 2004; Johnson et al., 1994). Increased CO_2 led to a shift in the microbial community composition, with more fungal microbes and less bacterial microbes present (Jin, 2010). Increased warming and increased nitrogen deposition left total soil carbon the same, however it decreased the microbial contribution to carbon storage (Liang et al, 2012).

Warming and drying (aeration) both increased CO_2 emissions, but this did not correlate with measures of microbial activity, suggesting that the quality of the peat and the presence of microbial inhibitors (such as a lower pH, the peat substrate, and the

presence of microbial inhibitors) has a bigger impact on microbial carbon cycling (Preston et al, 2012). A microbial study in a grassland found that warming has less of an impact on the soil microbial functions and enzymes than plant diversity (Steinauer et al, 2015).

Caveats

The absence or decrease in bioindicator abundance within the peat depth profile reflects the decrease in total microbial abundance. This could mean the bioindicators were of too low abundance to be measured in the deeper layers of the profile, resulting in incomplete depth profiles and $\delta^{13}C$ profiles. However, the comparatively higher abundance of fungi allowed for more complete data of its $\delta^{13}C$ signature.

CHAPTER TWO CONCLUSION AND FUTURE WORK

The feedback mechanisms behind the biogeochemical cycling of peatlands are complex and are based on many environmental factors. Understanding the role of the microbial community in carbon cycling in these ecosystems and their response to climatic manipulation is a crucial component.

This analysis used the June 2015 sampling date to investigate the response of the microbial community and carbon use to deep peat warming. The analysis compliments other microbial ecology findings from the SPRUCE project and sets the stage for future microbial work. Significant responses from the microbial community to the warming

could indicate vulnerability of the peatland ecosystem to climate change, and responsiveness to temperature especially in the surface of the peat profile. The warming decreased the abundance of actinomycetes in the surface of the peat profile, and increased the relative abundance of the fungal community. Fungal carbon use indicated an initial increase in the use of new carbon in response to the warming. Longer term warming could lead to loss of soil carbon storage from the peatland, although it appears initially the microbial community is using the newer carbon sources that are produced because of the warming treatment.

Continued deep peat warming along with surface warming and addition of CO_2 could have more evident effects on microbial carbon use in the peatland. This analysis adds to the pool of knowledge about peatlands and carbon cycling, contributing important microbial data. Microbial information can eventually be incorporated into climate models to give a more complete and accurate prediction of the response of northern peatlands to climate change which will be of critical importance for planning and scientific purposes. Continuing to study the long term response of the microbial community to climatic manipulation will be essential in order to understand the actual response of the carbon cycling to climate change within peatland ecosystems.

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APPENDIX A

δ^{13}CPLFA Method Development

The PLFA extraction method was modified for peat based on the method of extraction from soil. Modifications were found to be necessary due to the high organic matter content of peat and low microbial abundance. The amount of peat material needed for extraction was determined by analyzing peat cores taken from the same sample site in years previous. The amount of material needed for analysis increased drastically compared to the soil extraction method; from .5g of soil to 2g, 6-8g, and 10g of peat depending on the depth increment.

Extra cleaning steps were needed including extra centrifuging steps during extraction of total lipid content. Increased volume of collection of final lipid fraction was necessary due to low microbial abundance. Due to high organic content, the size of silica columns was found necessary to increase from .5g square columns to 2g round and 6g round silica columns depending on the depth increment. A ratio of 2g of peat extraction (from the original dry material used) to a 6g silica column during phospholipid separation was necessary due to high organic content. Forcing too much of the lipid fraction volume flooded the silica columns, leading to inaccurate and incomplete results.

The GC-IRMS analysis required method development within the program in regards to timing (Jess Gutknecht's work). Naming the lipids also required method development. Each sample's chromatogram was visually evaluated and hand named based on the M1M2 standard and compared to the FAME Standard retention time. Jess's FAME standard retention time was adjusted based on the machine's run time and

variance. The GC-IRMS displayed temporal variance over time which had to be accounted for. Lastly, the peat chromatograms displayed extra small peaks of organic material to be deciphered between the lipids due to high organic material.

APPENDIX B

Non-parametric Statistics for Chapter One Exploratory Analysis

Due to unequal variance in the raw data, non-parametric approaches could also be effective for the exploratory statistical analysis in the first chapter. Transforming the data to make the variability more similar was one technique used. The log value of the total microbial abundance was taken to compare the depth increments with unequal variance (Figure 42, 43). It also allowed for better comparison of the raw microbial abundance data by temperature (Figure 44). Another technique was to use non-parametric correlation techniques such as Kendall's T or Spearman's P in JMP (Table 9). One further suggestion would be to add interactions into a multivariate model. This area of non-parametric statistical analysis could be part of further work in this research.

TABLES

Abundance (June 2014, September 2014, June 2015 Ambient)																				
	Total			Fungi			G- Bac			G+ Bac			Actino			Anaerobic Bac			Protozoa		
	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-
Depth	**<.0001**	**34.89**	-	**<.0001**	**10.13**	-	**<.0001**	**8.39**	-	**<.0001**	**11.52**	-	**<.0001**	**7.72**	-	**.0251**	**3.305**	-	**<.0001**	**14.33**	-
Sample Date	.0565	2.56	N/A	.3529	1.098	N/A	.6572	.5381	N/A	.0923	2.190	N/A	.1999	1.57	N/A	**.0211**	**3.45**	**N**	.6079	.5056	N/A
Sample Date at Depth Increment																					
0-20 cm	**.0038**	**5.77**	**N**	.0619	2.703	N/A	.4665	.8705	N/A	.0044	5.29	N	**.0115**	**4.36**	**N**	.1553	1.907	N/A	N/A	N/A	N/A
150-200 cm	**.0208**	**3.98**	**N**	.6249	.2797	N/A	.3466	1.490	N/A	.1543	2.374	N/A	.5743	.595	N/A	N/A	N/A	N/A	.1961	2.11	N/A
Temperature	**<.0001**	**23.67**	**+**	**.0021**	**9.89**	**+**	**.0128**	**6.41**	**+**	**.0008**	**11.74**	**+**	**.0019**	**10.0447**	**N/A**	.6397	.2210	N/A	.1890	1.7993	N/A
Temperature at Depth Increment at Sample Date																					
June 2014 0-20 cm	.9064	.0150	N/A	**.0208**	**7.5055**	-	**.0276**	**6.638**	-	**.0105**	**9.866**	-	**.0074**	**11.182**	-	**.0064**	**12.466**	-	N/A	N/A	N/A
June 2014 50-100 cm	**.0363**	**5.84**	**+**	.0794	4.4529	N/A	**.0470**	**5.498**	**+**	**.0092**	**10.37**	**+**	.2019	1.89	N/A	**.0140**	**69.91**	**+**	.6895	.1847	N/A
Water Content	**<.0001**	**20.69**	**+**	**<.0001**	**23.33**	**+**	**<.0001**	**19.54**	**+**	**<.0001**	**23.04**	**+**	**<.0001**	**16.74**	**+**	**.0164**	**6.04**	**+**	.2004	1.7071	N/A
Water Content at Depth																					
0-20 cm	**.0361**	**4.87**	-	.7018	.1419	N/A	.6283	.2386	N/A	.6859	.1663	N/A	.4833	.5030	N/A	N/A	N/A	N/A	.2874	1.177	N/A
Bulk Density	**<.0001**	**92.31**	-	**<.0001**	**20.43**	-	**<.0001**	**22.14**	-	**<.0001**	**25.39**	-	**<.0001**	**16.79**	-	.1978	1.68	N/A	**.0016**	**11.79**	-
Bulk Density at Depth Increment																					
0-20 cm	**.0215**	**5.90**	**+**	**<.0001**	**64.41**	**+**	**<.0001**	**22.82**	**+**	**<.0001**	**50.76**	**+**	**<.0001**	**50.2**	**+**	**<.0001**	**84.25**	**+**	N/A	N/A	N/A
200-250 cm	.0870	3.53	-	.2694	1.480	-	**.0303**	**15.05**	-	**.0092**	**14.28**	-	.1792	2.12	-	N/A	N/A	N/A	.2647	1.51	-

N/A= Insufficient Data

Table 1. The abundance table contains all the significant univariate analysis findings: p values, f values, and direction of relationship. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables for the sampling dates June 2014, September 2014, and June 2015 ambient plots. Sampling date significance that suggests natural variation is marked by an N. Statistical tests were only included in this table, if at least one variable had a significant result.

Relative Abundance (June 2014, September 2014, June 2015 Ambient)																		
	Fungi			G- Bac			G+ Bac			Actino			Anaerobic Bac			Protozoa		
	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-
Depth	**<.0001**	**44.47**	-	**<.0001**	**9.33**	**N**	**.0210**	**2.78**	+	**<.0001**	**9.22**	+	**<.0001**	**26.01**	-	N/A	N/A	N/A
Sample Date	.1259	1.947	N/A	**<.0001**	**9.33**	**N**	.3462	1.113	N/A	**.0146**	**3.71**	**N**	.4354	.9207	N/A	.4354	.8534	N/A
Sample Date at Depth Increment																		
20-50 cm	.0731	2.662	N/A	**.0022**	**7.08**	**N**	.1371	2.0438	N/A	.5703	.6839	N/A	.4214	1.0058	N/A	.8408	.0440	N/A
Month	.2604	1.279	N/A	.4274	.6347	N/A	.8188	.0527	N/A	**.0468**	**4.03**	**N**	.3614	.8437	N/A	**.1979**	**1.7269**	**N**
Temperature at Sample Month																		
June	**<.0001**	**25.39**	+	.3153	1.0231	N/A	.8152	.0550	N/A	**.0003**	**13.74**	-	**.0005**	**13.45**	-	**.0486**	**4.19**	-
Temperature at Depth Increment at Sample Date																		
Sept 2014 0-20 cm	.4690	.5665	N/A	.6203	.2614	N/A	.1140	2.9988	N/A	**.0216**	**7.3935**	-	.7842	.0802	N/A	N/A	N/A	N/A
June 2014 20-50 cm	.6301	.2627	N/A	.7363	.1303	N/A	.6147	.2816	N/A	**.0390**	**6.0759**	-	N/A	N/A	N/A	.9905	.0002	N/A
Water Content	**<.0001**	**58.97**	+	**.0004**	**13.24**	+	**.0231**	**5.52**	-	**.0002**	**14.62**	-	**.0101**	**6.98**	-	**.0193**	**8.10**	+
Bulk Density	**<.0001**	**58.97**	+	.3986	.7183	N/A	**.0119**	**6.51**	+	**.0004**	**13.19**	+	**<.0001**	**40.95**	+	.2766	1.2239	N/A

N/A= Insufficient Data

Table 2. The relative abundance table contains all the significant univariate analysis findings: p values, f values, and direction of relationship. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables for the sampling dates June 2014, September 2014, and June 2015 ambient plots. Sampling date significance that suggests natural variation is marked by an N. Statistical tests were only included in this table, if at least one variable had a significant result.

$\delta^{13}C$ (June 2014, September 2014, June 2015 Ambient)																				
	Total			Fungi			G- Bac			G+ Bac			Actino			Anaerobic Bac			Protozoa		
	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-
Depth	**<.0001**	**17.75**	**+**	**.0008**	**4.50**	**+**	**.0003**	**8.96**	**-**	.1376	1.7168	N/A	**.0464**	**2.44**	**-**	**.0059**	**4.08**	**-**	**.0009**	**11.72**	**+**
Sample Date	**.0102**	**3.89**	**N**	.1773	1.6812	N/A	.1980	1.5985	N/A	.3903	1.0127	N/A	.2820	1.3057	N/A	.1156	2.0661	N/A	.7850	.0776	N/A
Sample Date at Depth Increment																					
200-250 cm	.3442	.9636	N/A	.4407	.6358	N/A	.0202	9.8303	N/A	**.0427**	**5.14**	**N**	.8548	.0365	N/A	.3714	.8685	N/A	N/A	N/A	N/A
Month	**.0041**	**8.49**	**N**	.2604	1.2791	N/A	.4274	.6347	N/A	.8188	.0527	N/A	**.0468**	**4.0351**	**N**	.3614	.8437	N/A	.1979	1.7269	N/A
Temperature	**.0018**	**10.06**	**-**	**.0054**	**8.16**	**-**	.4214	.6542	N/A	.4942	.4707	N/A	.2848	1.16	N/A	.2846	1.16	N/A	**.0276**	**6.15**	**-**
Temperature at Depth Increment																					
50-100 cm	**.0418**	**4.507**	**-**	.2063	1.8042	N/A	**.0416**	**5.4598**	**-**	.7262	.1260	N/A	.8271	.0567	N/A	.1667	13.91	N/A	**.0114**	**31.13**	**-**
Temperature at Depth Increment at Sample Date																					
Sept 2014 0-20 cm	**.0466**	**5.15**	**+**	.8830	.0228	N/A	**.0391**	**5.82**	**+**	.2314	1.6236	N/A	.1216	2.9985	N/A	.2794	1.3746	N/A	N/A	N/A	N/A
June 2014 20-50 cm	.2896	1.265	N/A	**.0225**	**802.54**	**-**	.6246	.4476	N/A	.4005	.9550	N/A	.0697	12.8605	N/A	N/A	N/A	N/A	.9336	.0110	N/A
June 2014 200-250 cm	.6505	.2272	N/A	.8266	.0524	N/A	**.0314**	**409.6**	**-**	.4589	.6263	N/A	.5183	.8912	N/A	.3752	.9468	N/A	N/A	N/A	N/A
Water Content	**<.0001**	**19.1711**	**-**	**.0012**	**11.26**	**-**	.2676	1.2491	N/A	.9023	.0152	N/A	**.0216**	**5.58**	**-**	**.0404**	**4.92**	**-**	.1099	2.9451	N/A
Bulk Density	.0357	4.4847	N/A	**.0046**	**8.49**	**+**	**.0138**	**6.38**	**-**	.0727	3.287	N/A	.5489	.3637	N/A	.1486	2.14	N/A	.4051	.7404	N/A

N/A= Insufficient Data

Table 3. The $\delta^{13}C$ table contains all the significant univariate analysis findings: p values, f values, and direction of relationship. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables for the sampling dates June 2014, September 2014, and June 2015 ambient plots. Sampling date significance that suggests natural variation is marked by an N. Statistical tests were only included in this table, if at least one variable had a significant result.

Multivariate Statistics					
		NMDS 1	NMDS 2	r^2	p
Abundance NMDS	Depth	-0.48996	0.87174	0.2389	0.000999
	Temperature	-0.24612	0.96924	0.1353	0.000999
	Bulk Density	0.70763	-0.70658	0.2152	0.000999
	Water Content	-0.52283	0.85244	0.2784	0.000999
Relative Abundance NMDS	Depth	0.011538	-0.999930	0.1390	0.000999
	Temperature	-0.010121	-0.999950	0.0512	0.009990
	Bulk Density	0.289982	0.957030	0.0964	0.000999
	Water Content	-0.187231	-0.982320	0.1687	0.000999
$\delta^{13}C$ NMDS	Depth	-0.43151	0.90211	0.0067	0.56144
	Temperature	0.38916	0.92117	0.0085	0.47453
	Bulk Density	-0.13910	0.99028	0.0004	0.97902
	Water Content	0.88290	0.46955	0.46955	0.00999

Table 4. Multivariate Statistics for Abundance, Relative Abundance, and $\delta^{13}C$ NMDS Ordinations.

Abundance (June 2015)																					
	Total			Fungi			G- Bac			G+ Bac			Actino			Anaerobic Bac			Protozoa		
	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-
Sample Date at Depth Increment																					
0-20 cm	**.0038**	**5.77**	**J**	.0619	2.7036	N/A	.4665	.8705	N/A	**.0044**	**5.29**	**J**	**.0115**	**4.3622**	**J**	.1553	1.9007	N/A	N/A	N/A	N/A
Depth	**<.0001**	**10.97**	**-**	**.0002**	**13.62**	**-**	.0633	3.2610	N/A	**.0064**	**6.77**	**-**	.1201	2.316	-	.1029	2.8160	N/A	N/A	N/A	N/A
Temperature Depth Increment																					
0-20 cm	.0540	4.0592	N/A	.5302	.4022	N/A	.9524	.0036	N/A	.6880	.1614	N/A	**.0480**	**6.7843**	-	.8839	.0217	N/A	N/A	N/A	N/A
Water Content	**.0033**	**10.00**	**+**	**.0366**	**5.05**	**+**	.1973	1.7928	N/A	**.0475**	**4.49**	**+**	**.0033**	**10.00**	**+**	**.0366**	**5.05**	**+**	N/A	N/A	N/A
Bulk Density	**<.0001**	**24.69**	**-**	**.0005**	**17.20**	**-**	**.0483**	**4.49**	**-**	**.0056**	**9.78**	**-**	.0674	3.8509	N/A	.1844	1.9839	N/A	N/A	N/A	N/A

J= June 2015 Standout Sample Date
N/A= Insufficient Data

Table 5. The Abundance (June 2015) table contains all the significant univariate analysis findings: p values, f values, and direction of relationship. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables for the June 2015 sampling date. Statistical tests were only included in this table, if at least one variable had a significant result.

δ13C (June 2015)																				
	Total			Fungi			G- Bac			G+ Bac			Actino			Anaerobic Bac			Protozoa		
	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-
Sample Date	**.0102**	**3.89**	J	.1773	1.6812	N/A	.1980	1.5985	N/A	.3903	1.0127	N/A	.2820	1.3057	N/A	.1158	2.0661	N/A	.7850	.0776	N/A
Depth	**.0014**	**5.84**	+	.0945	3.2962	N/A	.9875	.0003	N/A	.2573	1.5095	N/A	.8756	.0267	N/A	.5087	.4848	N/A	N/A	N/A	N/A
Depth At Sample Date																					
20-50 cm	**.0382**	**3.22**	J	.3264	1.2514	N/A	.5372	.7536	N/A	.7105	.4646	N/A	.2152	1.7234	N/A	.6994	.4861	N/A	N/A	N/A	N/A
50-100 cm	**.0246**	**3.62**	J	.4960	.7527	N/A	.2009	1.9285	N/A	N/A	N/A	N/A	.0757	7.1275	N/A	N/A	N/A	N/A	.1651	3.3401	N/A
Temperature	.1739	1.933	N/A	**.0171**	**7.65**	-	.9826	.0005	N/A	.6707	.1886	N/A	.2786	1.4188	N/A	.1579	2.5001	N/A	N/A	N/A	N/A
Water Content	**.0064**	**8.50**	-	.3755	.8471	N/A	.8823	.0229	N/A	.6932	.1622	N/A	.4708	.5921	N/A	.4106	.7655	N/A	N/A	N/A	N/A
Bulk Density	**.0441**	**4.39**	+	.2335	1.5744	N/A	.9288	.0083	N/A	.4724	.5454	N/A	.9876	.0003	N/A	.7080	.1522	N/A	N/A	N/A	N/A

J= June 2015 Standout Sample Date
N/A= Insufficient Data

Table 6. The Relative Abundance (June 2015) table contains all the significant univariate analysis findings: p values, f values, and direction of relationship. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables for the June 2015 sampling date. Statistical tests were only included in this table, if at least one variable had a significant result.

δ13C (June 2015)																				
	Total			Fungi			G- Bac			G+ Bac			Actino			Anaerobic Bac			Protozoa		
	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-	p	f	+/-
Depth	**.0014**	**5.84**	+	.0945	3.2962	N/A	.9875	.0003	N/A	.2573	1.5095	N/A	.8756	.0267	N/A	.5087	.4848	N/A	N/A	N/A	N/A
20-50 cm	**.0382**	**3.22**	N/A	.3264	1.2514	N/A	.5372	.7536	N/A	.7105	.4646	N/A	.2152	1.7234	N/A	.6994	.4861	N/A	N/A	N/A	N/A
50-100 cm	**.0246**	**3.62**	N/A	.4960	.7527	N/A	.2009	1.9285	N/A	N/A	N/A	N/A	.0757	7.1275	N/A	N/A	N/A	N/A	.1651	3.3401	N/A
Temperature	.1739	1.933	N/A	**.0171**	**7.65**	-	.9826	.0005	N/A	.6707	.1886	N/A	.2786	1.4188	N/A	.1579	2.5001	N/A	N/A	N/A	N/A
Water Content	**.0064**	**8.50**	-	.3755	.8471	N/A	.8823	.0229	N/A	.6932	.1622	N/A	.4708	.5921	N/A	.4106	.7655	N/A	N/A	N/A	N/A
Bulk Density	**.0441**	**4.39**	+	.2335	1.5744	N/A	.9288	.0083	N/A	.4724	.5454	N/A	.9876	.0003	N/A	.7080	.1522	N/A	N/A	N/A	N/A

N/A= Insufficient Data

Table 7. The $\delta^{13}C$ (June 2015) table contains all the significant univariate analysis findings: p values, f values, and direction of relationship. Linear regression and Bivariate Oneway ANOVA analysis was used for dependent and independent variables for the June 2015 sampling date. Statistical tests were only included in this table, if at least one variable had a significant result.

Multivariate Statistics					
		NMDS 1	NMDS 2	r^2	p
June 2015 Abundance NMDS	Depth	0.45096	0.89255	0.8408	<0.001
	Temperature	0.07988	0.99680	0.2304	0.053846
	Bulk Density	-0.48273	-0.87577	0.6442	<0.001
	Water Content	0.30207	0.95329	0.5052	<0.001
June 2015 Relative Abundance	Depth	-0.84834	-0.52945	0.7119	<0.001
	Temperature	-0.87915	-0.47655	0.2788	0.016983
	Bulk Density	0.99556	0.09418	0.6355	<0.001
	Water Content	-0.85636	-0.51638	0.5358	<0.001
June 2015 $\delta^{13}C$ NMDS	Depth	-0.50783	-0.86146	0.1678	0.11089
	Temperature	-0.34931	-0.93701	0.3003	0.01299
	Bulk Density	0.61362	0.78960	0.0635	0.45954
	Water Content	-0.66263	-0.74895	0.1157	0.22078

Table 8. Multivariate Statistics for June 2015 Abundance, June 2015 Relative Abundance, and June 2015 $\delta^{13}C$ NMDS Ordinations.

Non-parametric Statistical Correlations				
Variable	By Variable	Spearman's P	Kendall T	p value
Temperature	Total Biomass		.2069	<.0001
Temperature	Total Biomass	.3069		<.0001
Temperature	Total $\delta^{13}C$		-0.1615	.0022
Temperature	Total $\delta^{13}C$	-0.2502		.0012

Table 9. Non-parametric Statistical Correlations

FIGURES

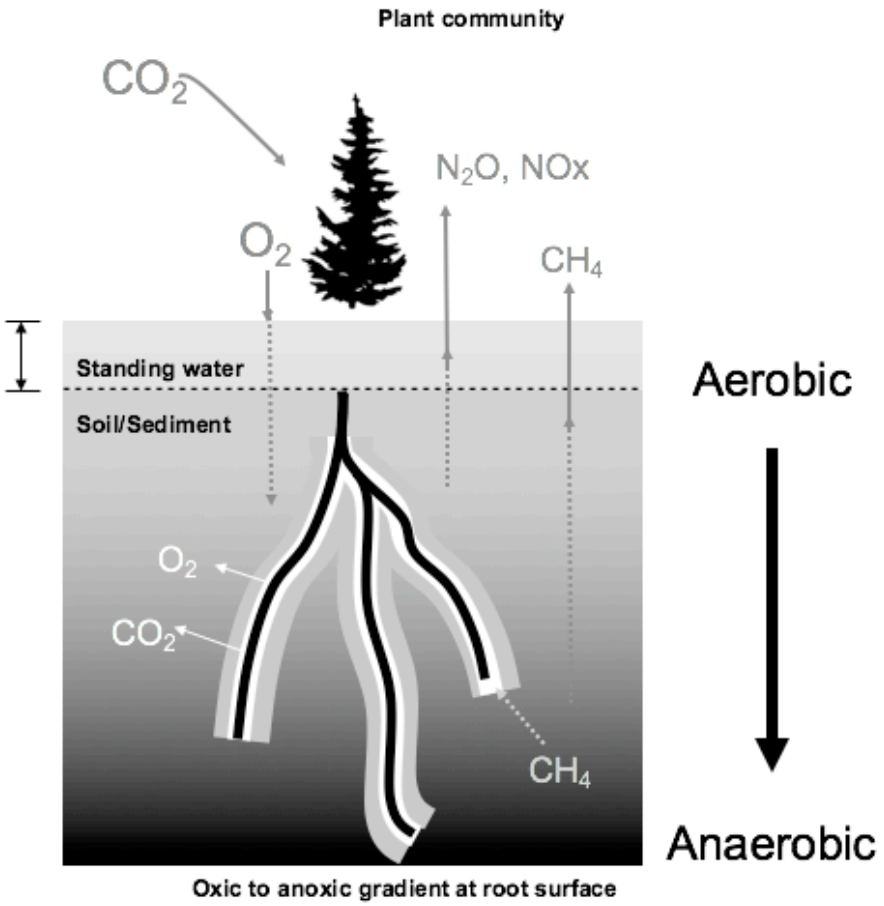

Figure 1. Wetland hydrology schematic (modified from Gutknecht et al, 2006). Depicting the oxic and anoxic conditions of the soil and sediment in wetland ecosystems, and the biogeochemical cycling that occurs under these conditions.

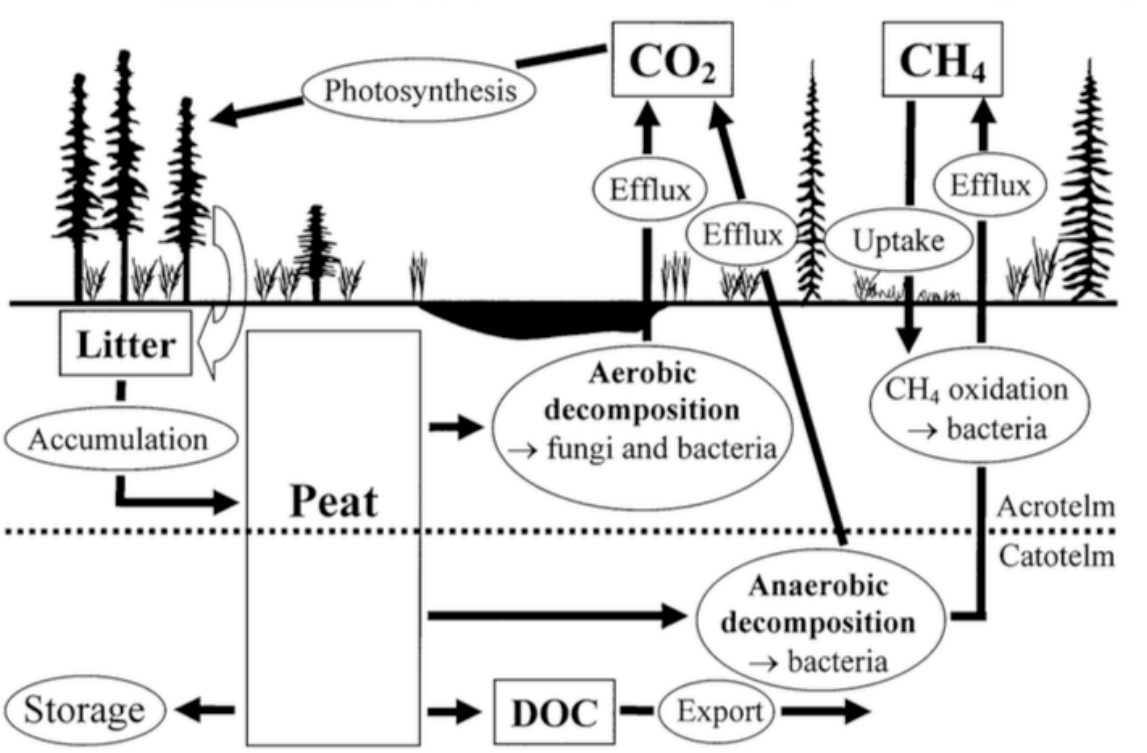

Figure 2. Major carbon pathways in peatlands and the microbial groups (fungi, bacteria) responsible for the dominant biogeochemical processes (Thormann, 2006)

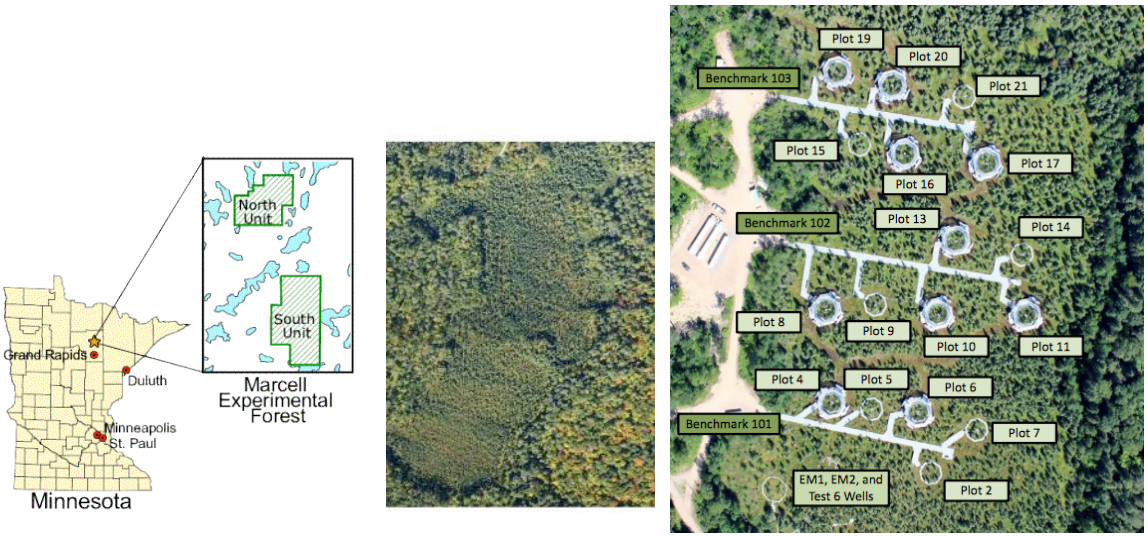

*Figure 3. **A** Site location of the SPRUCE experiment. **B** Aerial photo of the S1 bog. **C** Aerial photo of the S1 bog after construction of enclosures. (SPRUCE website)*

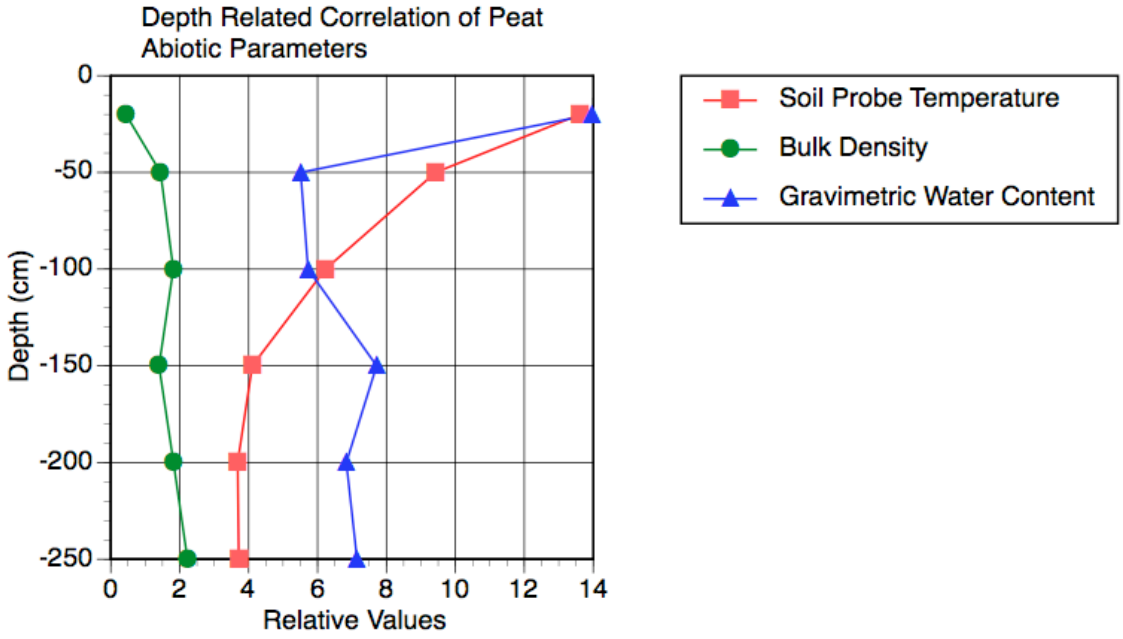

Figure 4. Autocorrelation of environmental variables with depth in June 2014, pre-treatment.

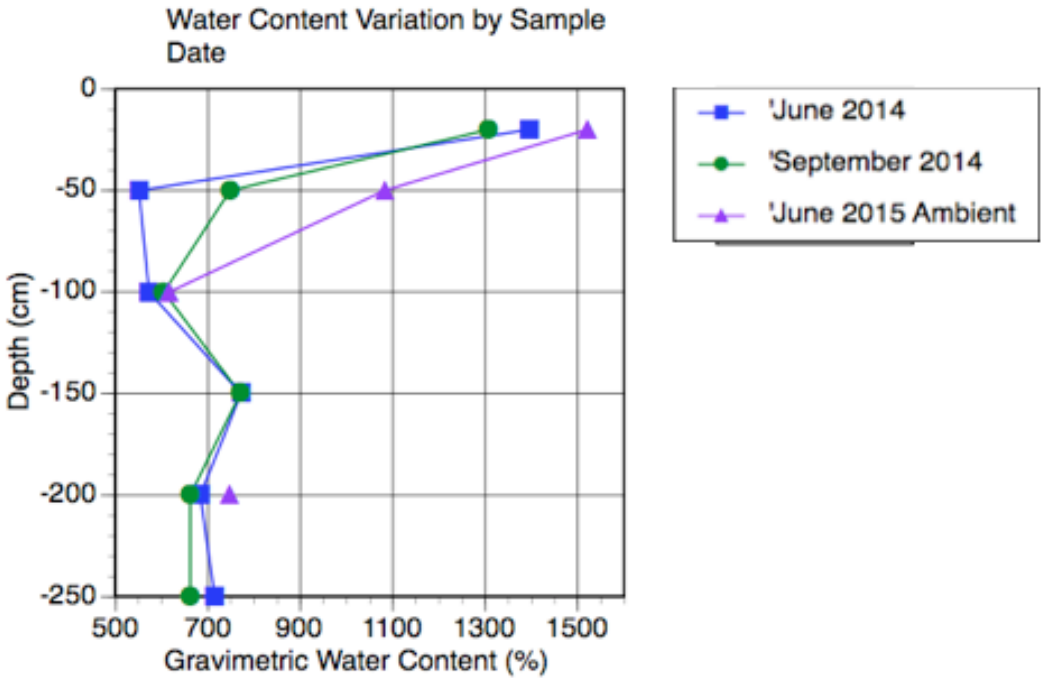

Figure 5. Gravimetric water content by depth for sampling dates June 2014, September 2014, and June 2015 Ambient plots. Average water content by depth is shown across 12 full plots, 2 partial, with 6 depth increments.

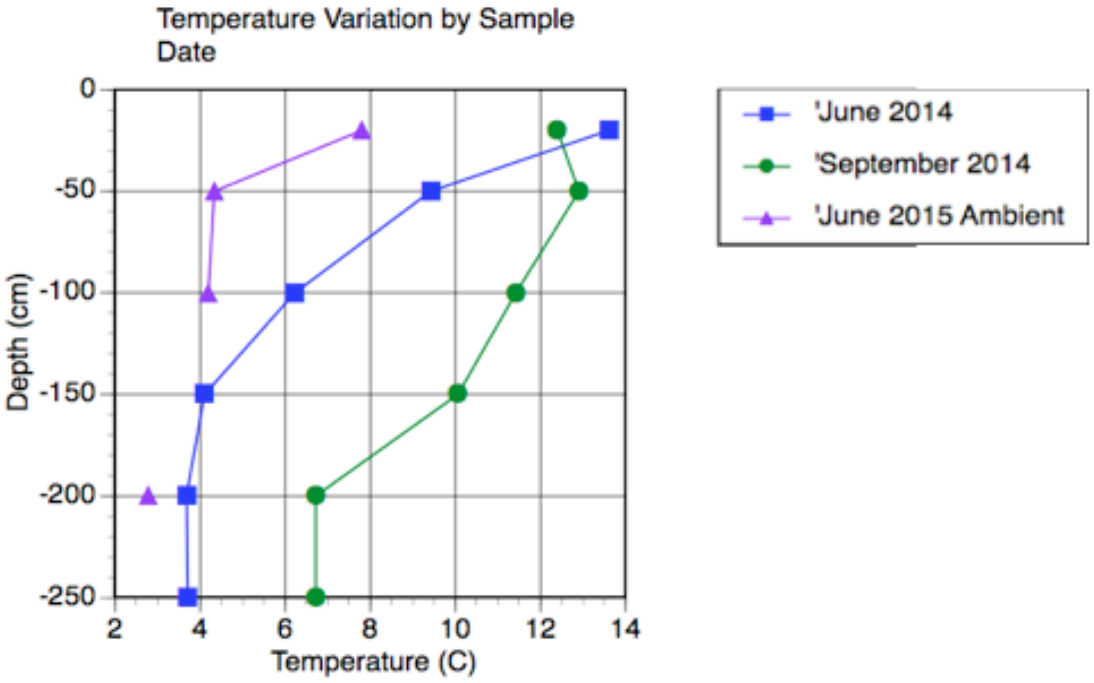

Figure 6. Soil temperature by depth for sampling dates June 2014, September 2014, and June 2015 Ambient plots. Average temperature by depth is shown across 12 full plots, 2 partial, with 6 depth increments.

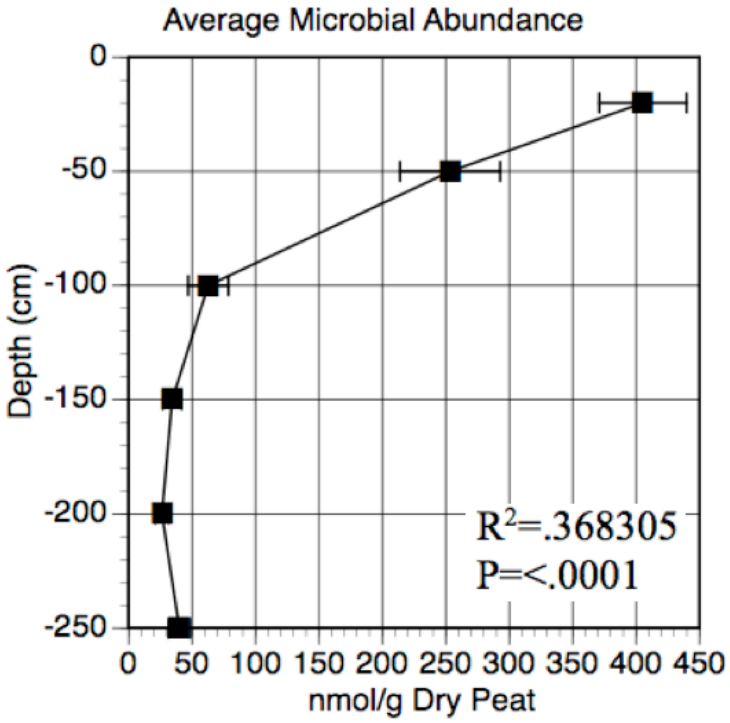

Figure 7. Total microbial abundance averaged across depth for the June 2014, September 2014, and June 2015 Ambient plots. Average microbial abundance by depth is shown across 12 full plots, 2 partial, with 6 depth increments, and across 3 sampling dates of June 2014, September 2014, and the June 2015 ambient plots. Standard error bars are shown.

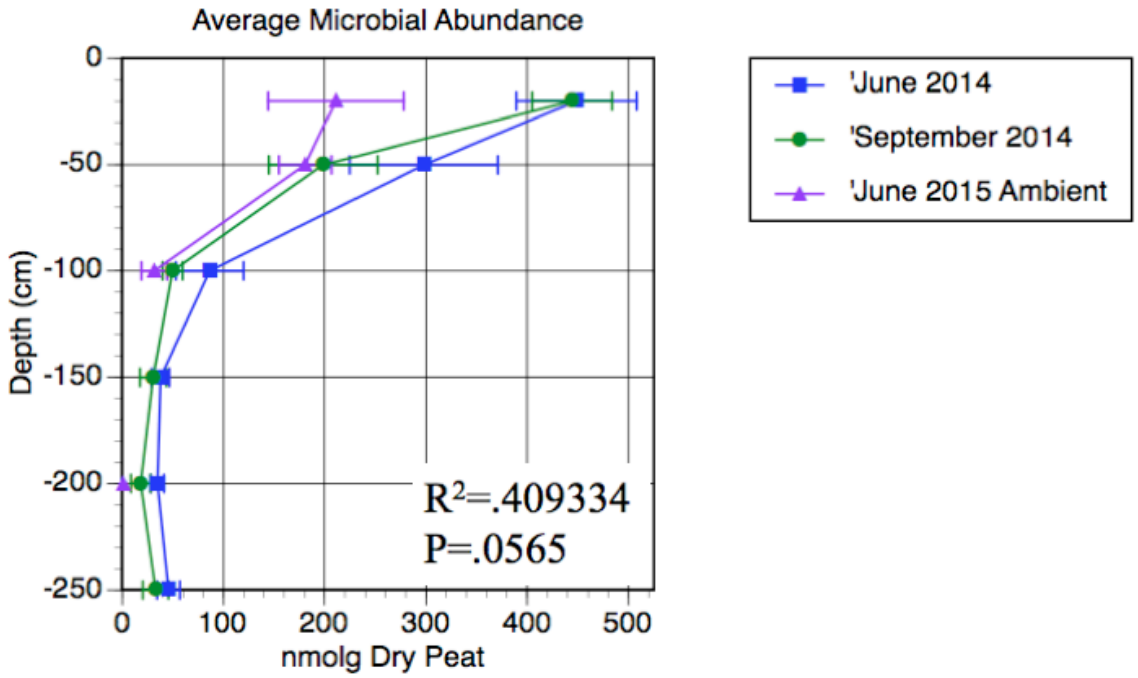

Figure 8. Average microbial abundance across depth by sample date. Average microbial abundance by depth is shown across 12 full plots, 2 partial, with 6 depth increments for 3 sampling dates. Standard error bars are shown.

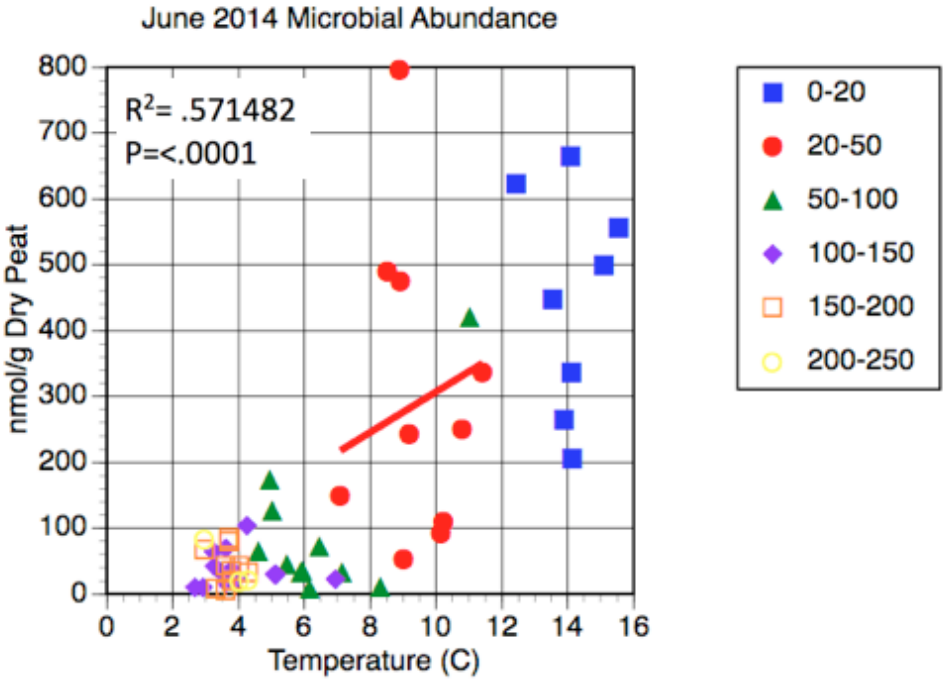

Figure 9. June 2014 total microbial abundance by temperature and depth increment. Each plot is shown and colored by depth increment. A positive relationship was significant in the 20-50cm depth increment.

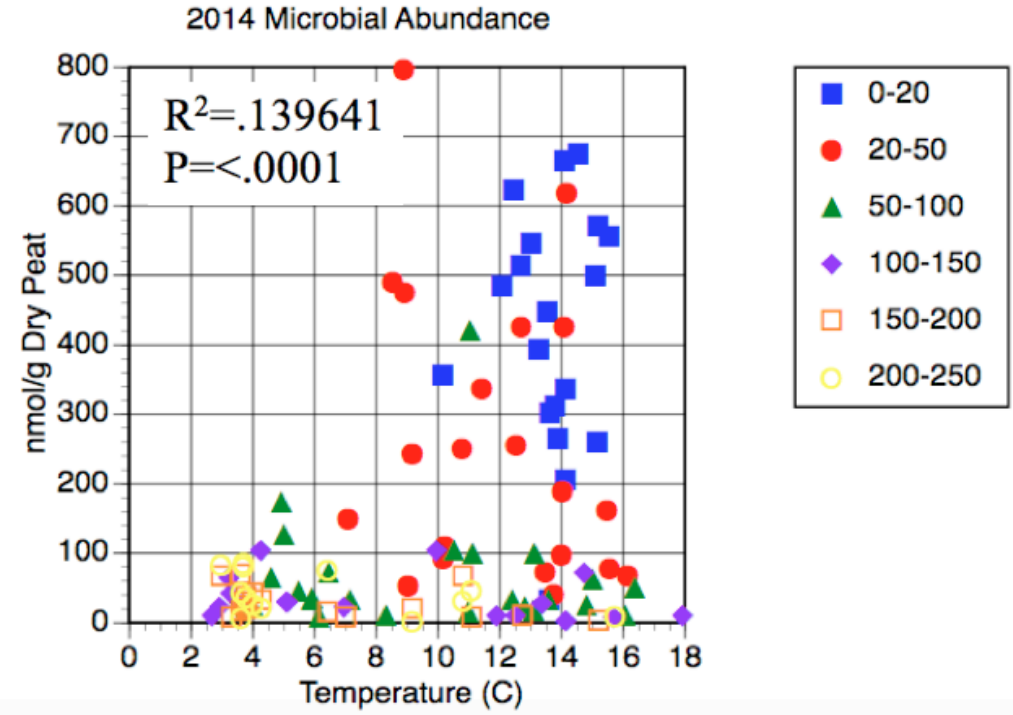

Figure 10. 2014 total microbial abundance by temperature and depth increment. Each plot is shown and colored by depth increment for the June and September 2014 sampling dates.

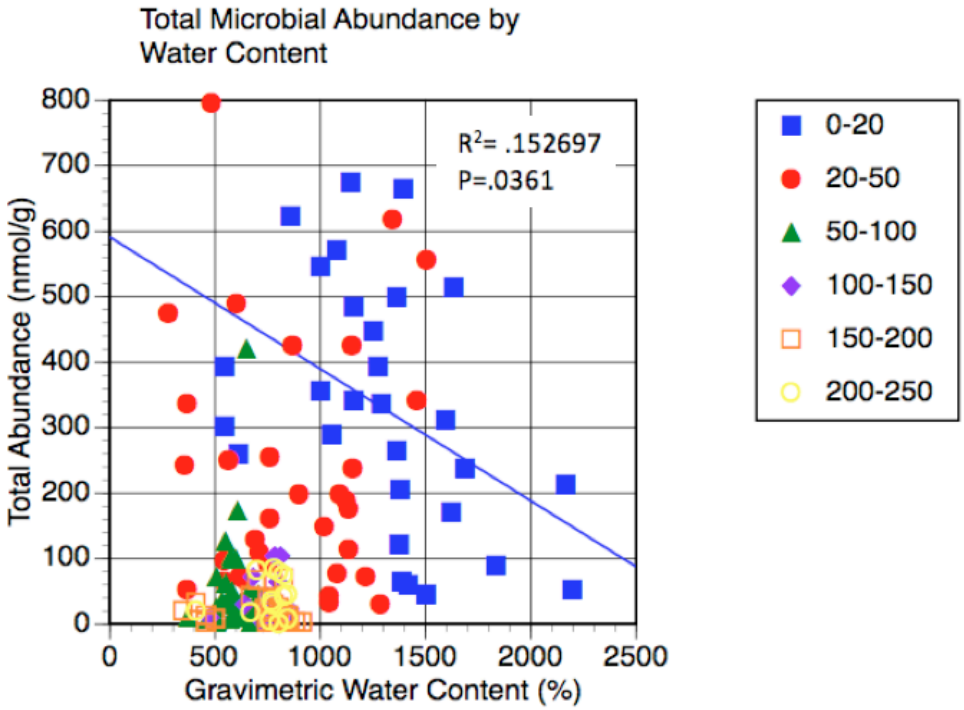

Figure 11. Total microbial abundance by water content. Each plot is shown and colored by depth increment across the sampling dates June 2014, September 2014, and June 2015 ambient plots. A negative relationship was significant in the 0-20cm depth increment.

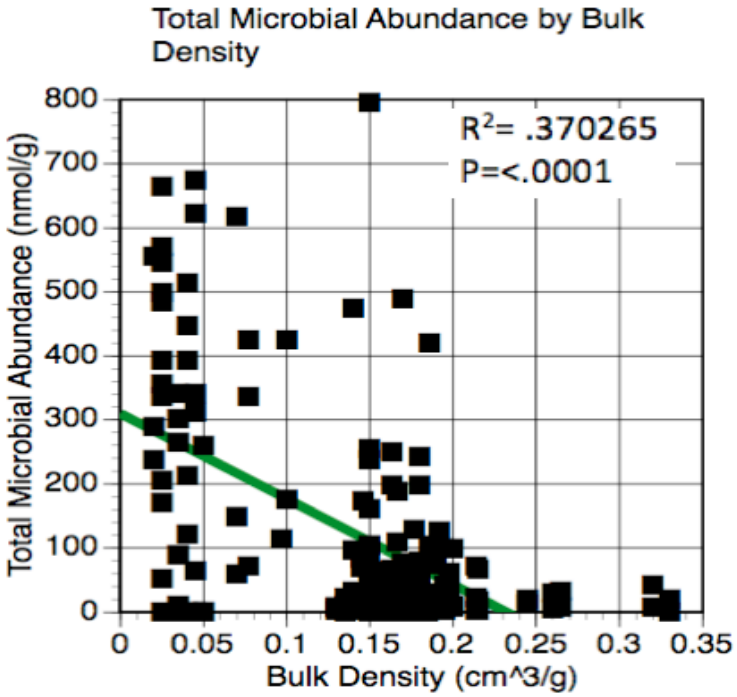

Figure 12. Total microbial abundance by bulk density. Bulk density measurements were taken in 2012 and microbial abundance data were based on off those measurements. A negative relationship across all depths was found.

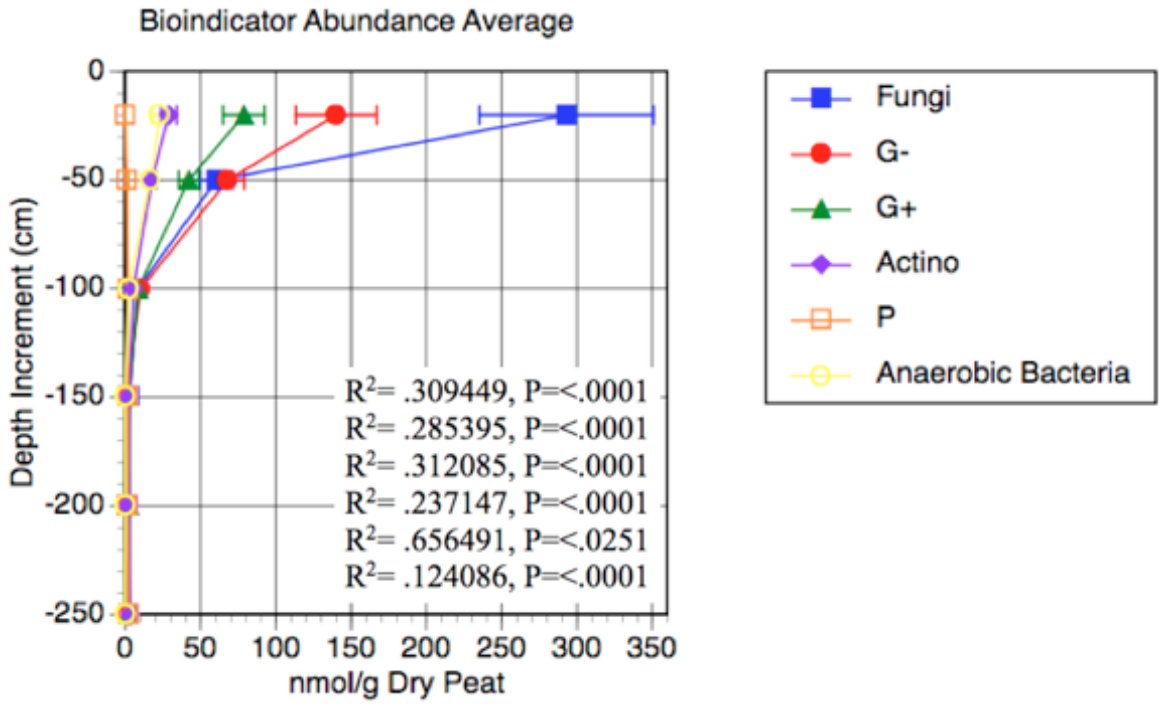

Figure 13. Average bioindicator abundance by depth for sample dates June 2014, September 2014, and June 2015 ambient plots. Average bioindicator abundance by depth is shown across 12 full plots, 2 partial, with 6 depth increments for 3 sampling dates. Standard error bars are shown.

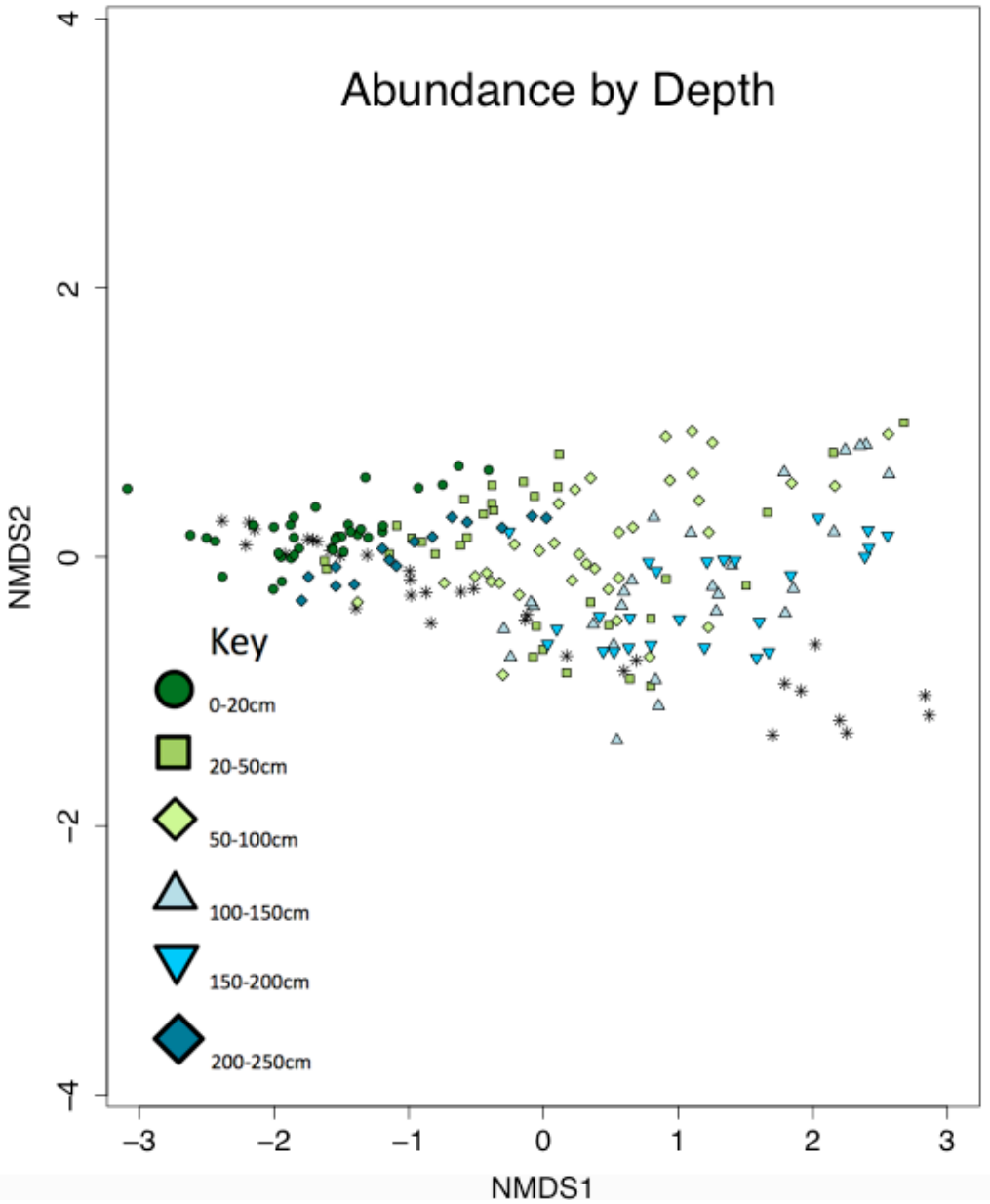

Figure 14. NMDS of Microbial Abundance by Depth, P<.0001 PERMANOVA analysis. The final stress value after 20 runs for this ordination was .073.

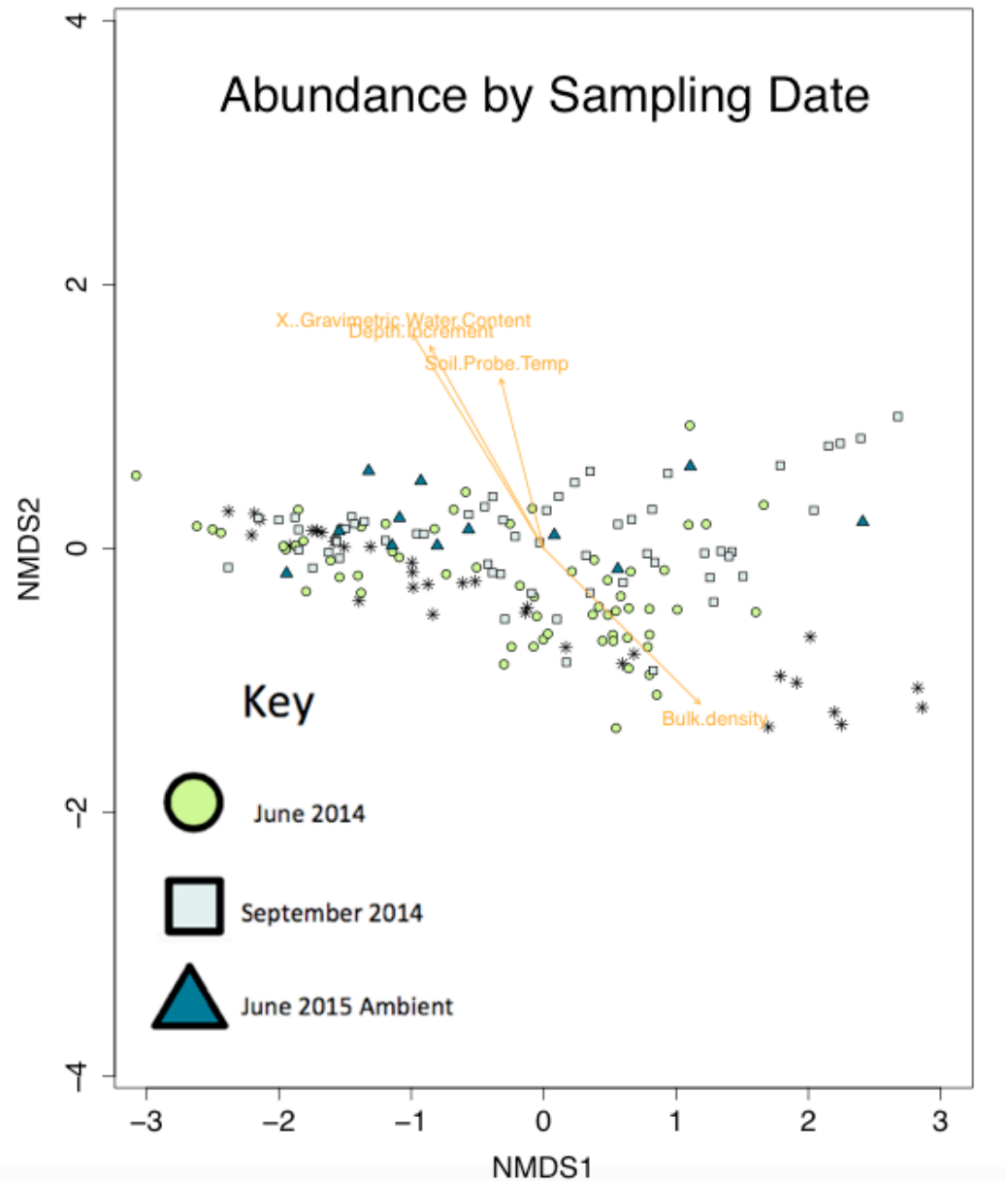

Figure 15. NMDS of Microbial Abundance by Sampling Date, P<.0001 PERMANOVA analysis. The final stress value after 20 runs for this ordination was .073.

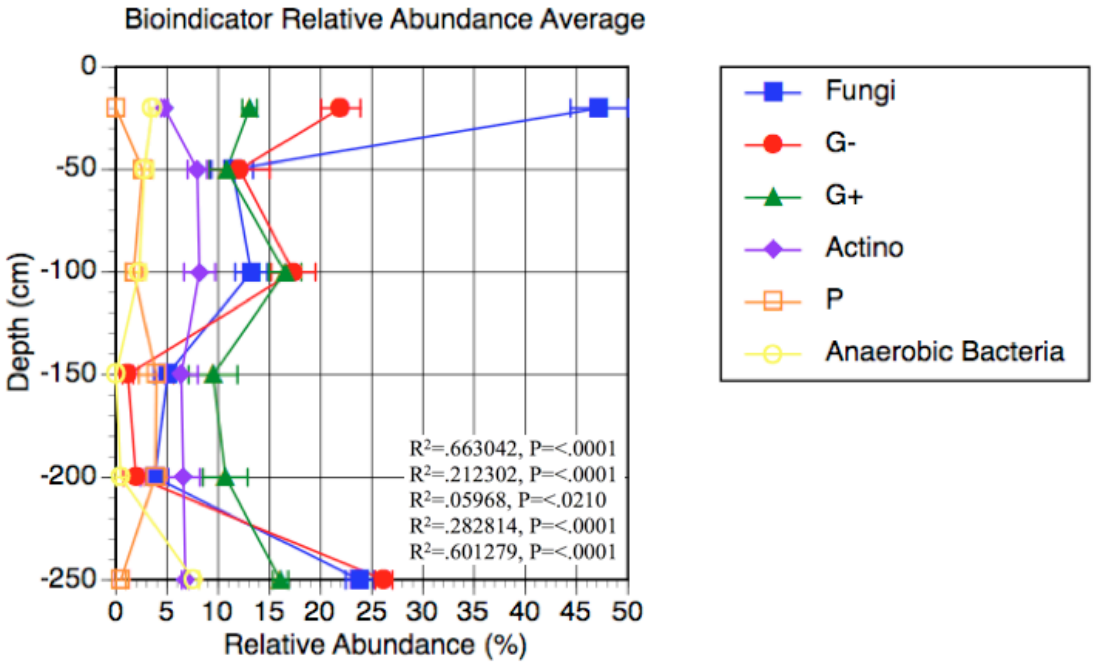

Figure 16. Bioindicator Relative Abundance averaged by depth. Average bioindicator relative abundance by depth is shown across 12 full plots, 2 partial, with 6 depth increments for 3 sampling dates. Standard error bars are shown.

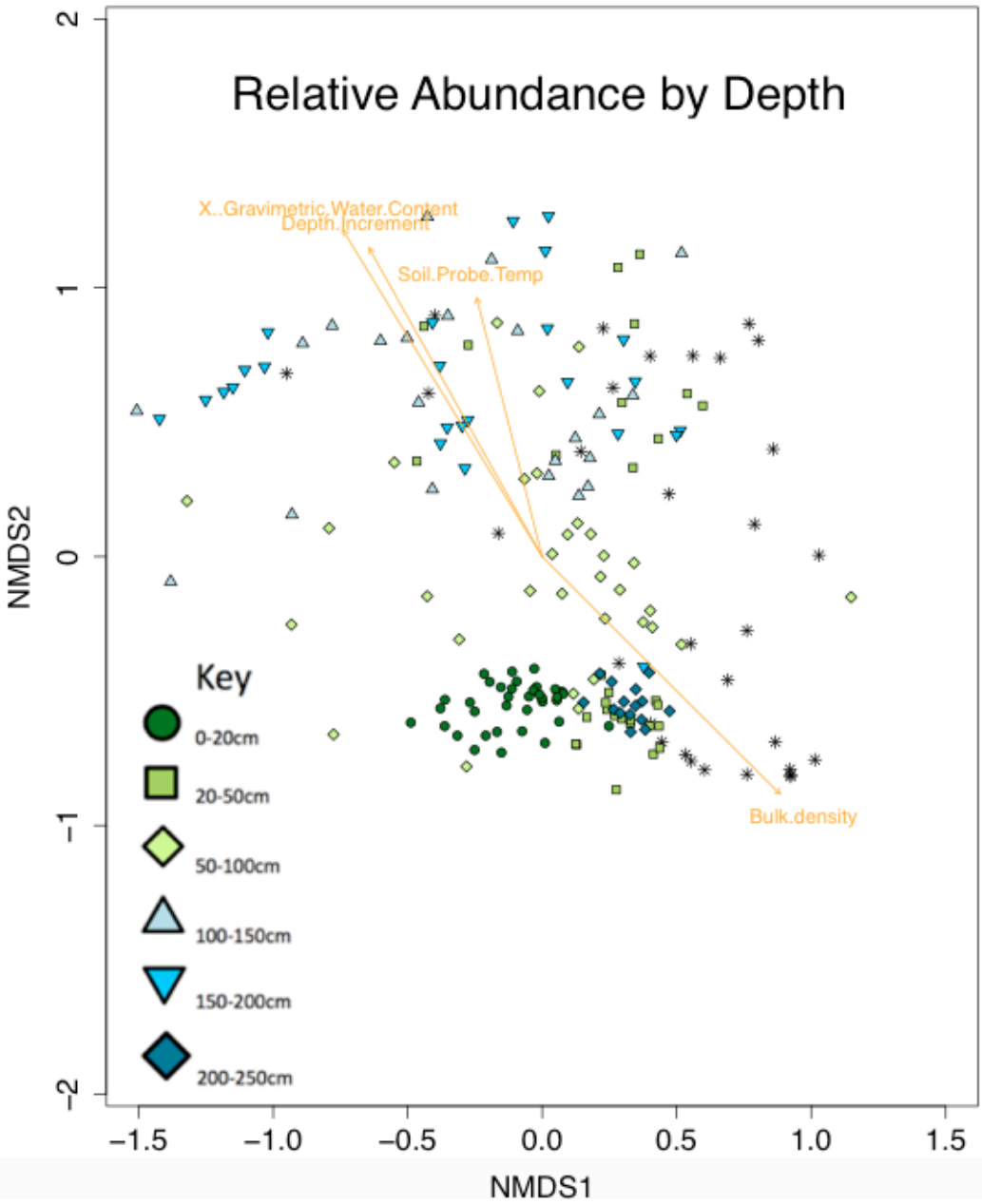

Figure 17. NMDS of Microbial Relative Abundance by Depth, P<.0001 PERMANOVA analysis. The final stress value after 20 runs for this ordination was .125.

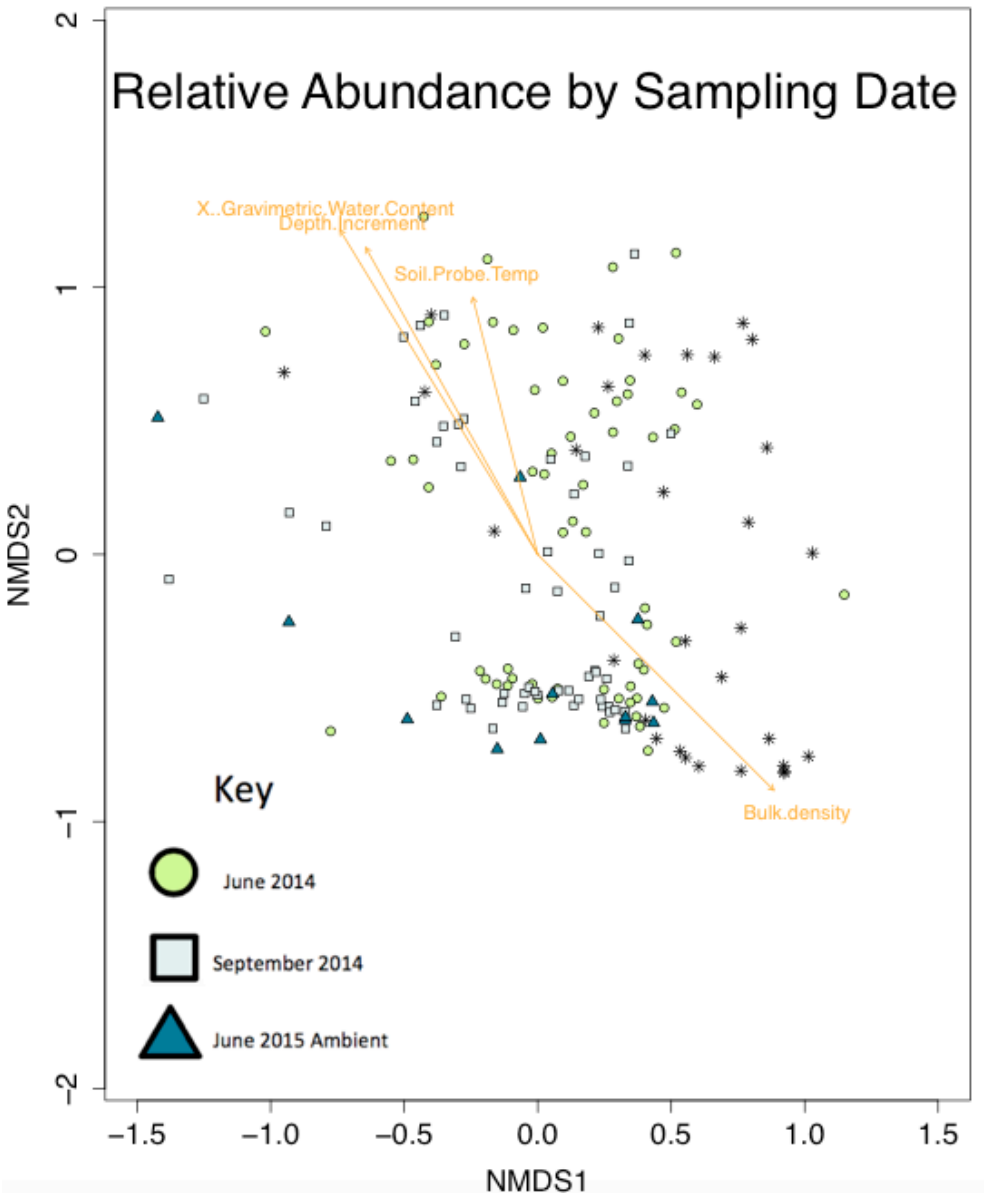

Figure 18. NMDS of Microbial Relative Abundance by Sampling Date, P<.0001 PERMANOVA analysis. The final stress value after 20 runs for this ordination was .125.

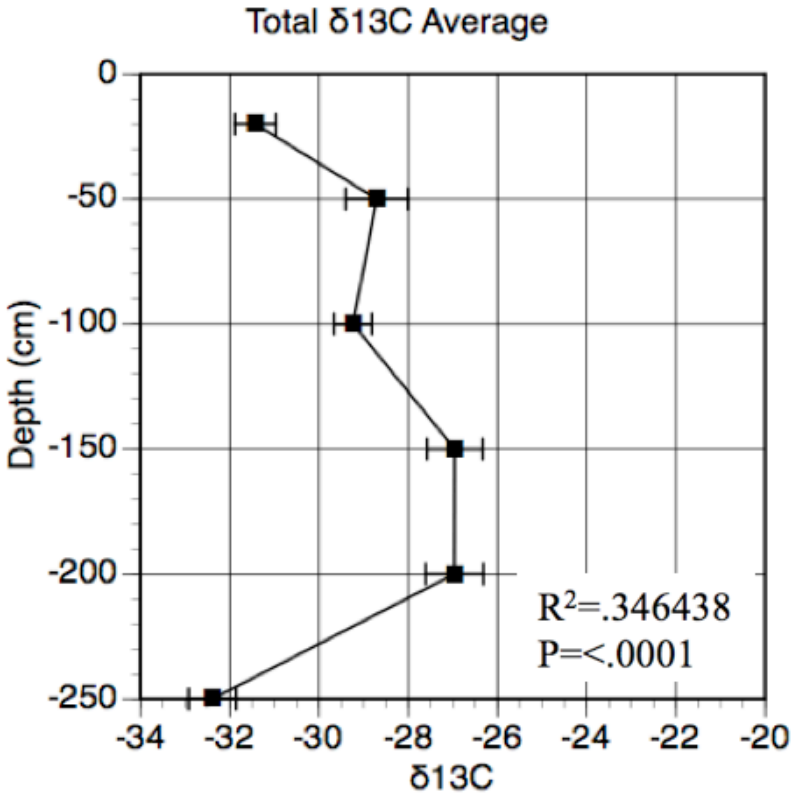

Figure 19. Total $\delta^{13}C$ values averaged across depths for the sampling dates June 2014 (all plots), September 2014 (all plots), and June 2015 (ambient plots only). Average total $\delta^{13}C$ by depth is shown across 12 full plots, 2 partial, with 6 depth increments for 3 sampling dates. Standard error bars are shown.

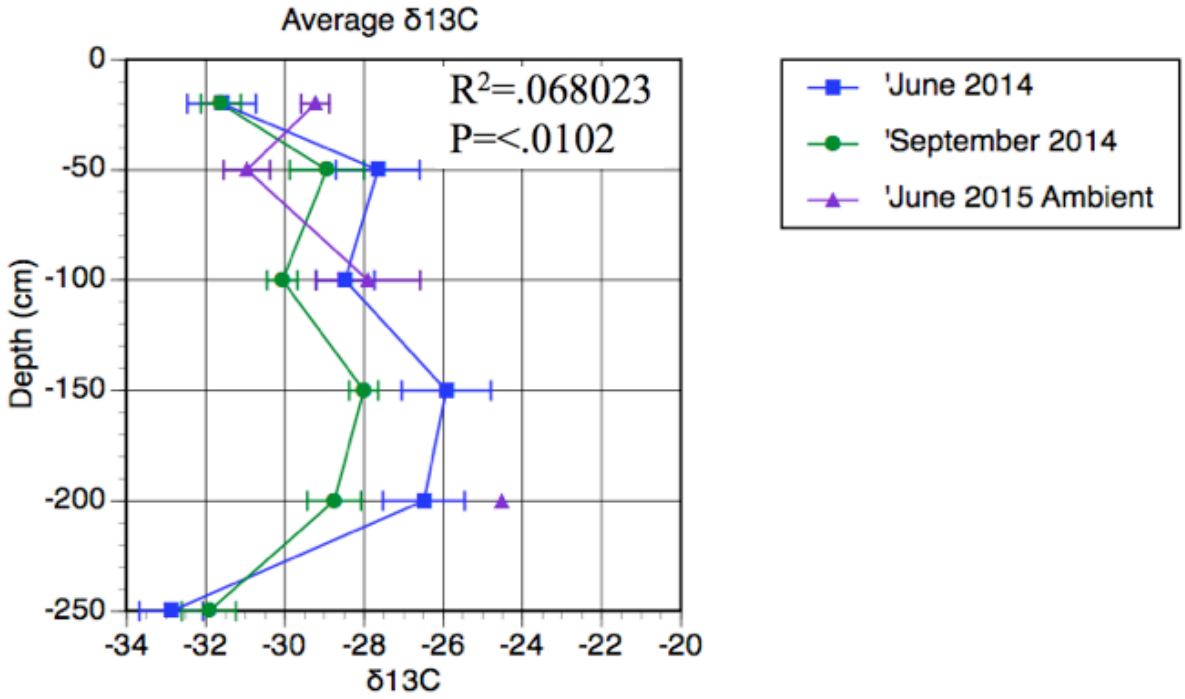

Figure 20. Average total $\delta^{13}C$ across depth by sample date. Average total $\delta^{13}C$ by depth and sample date is shown across 12 full plots, 2 partial, with 6 depth increments for 3 sampling dates. Standard error bars are shown.

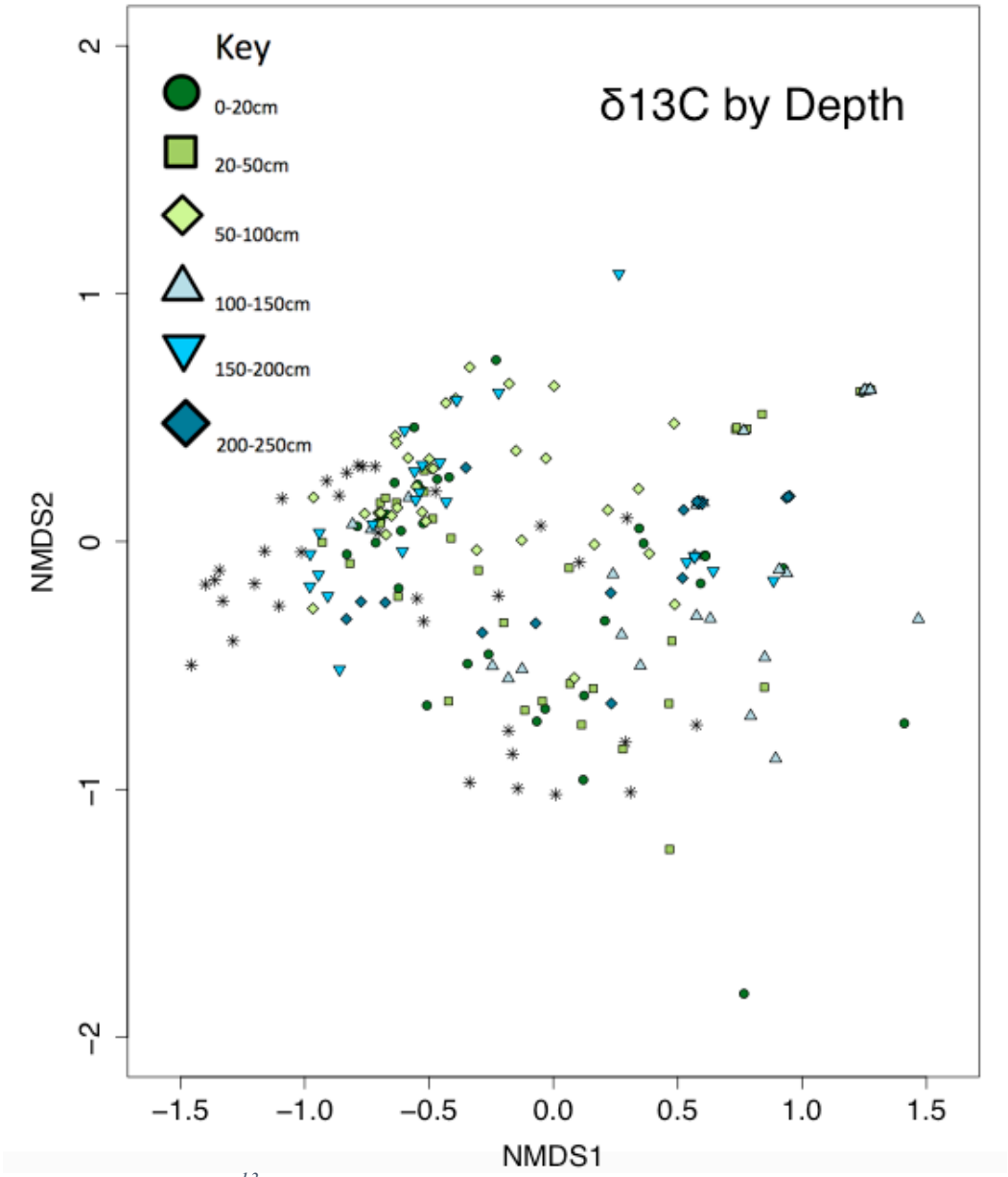

Figure 21. NMDS for $\delta^{13}C$ by depth, P<.0001 PERMANOVA analysis. The final stress value after 20 runs for this ordination is .136.

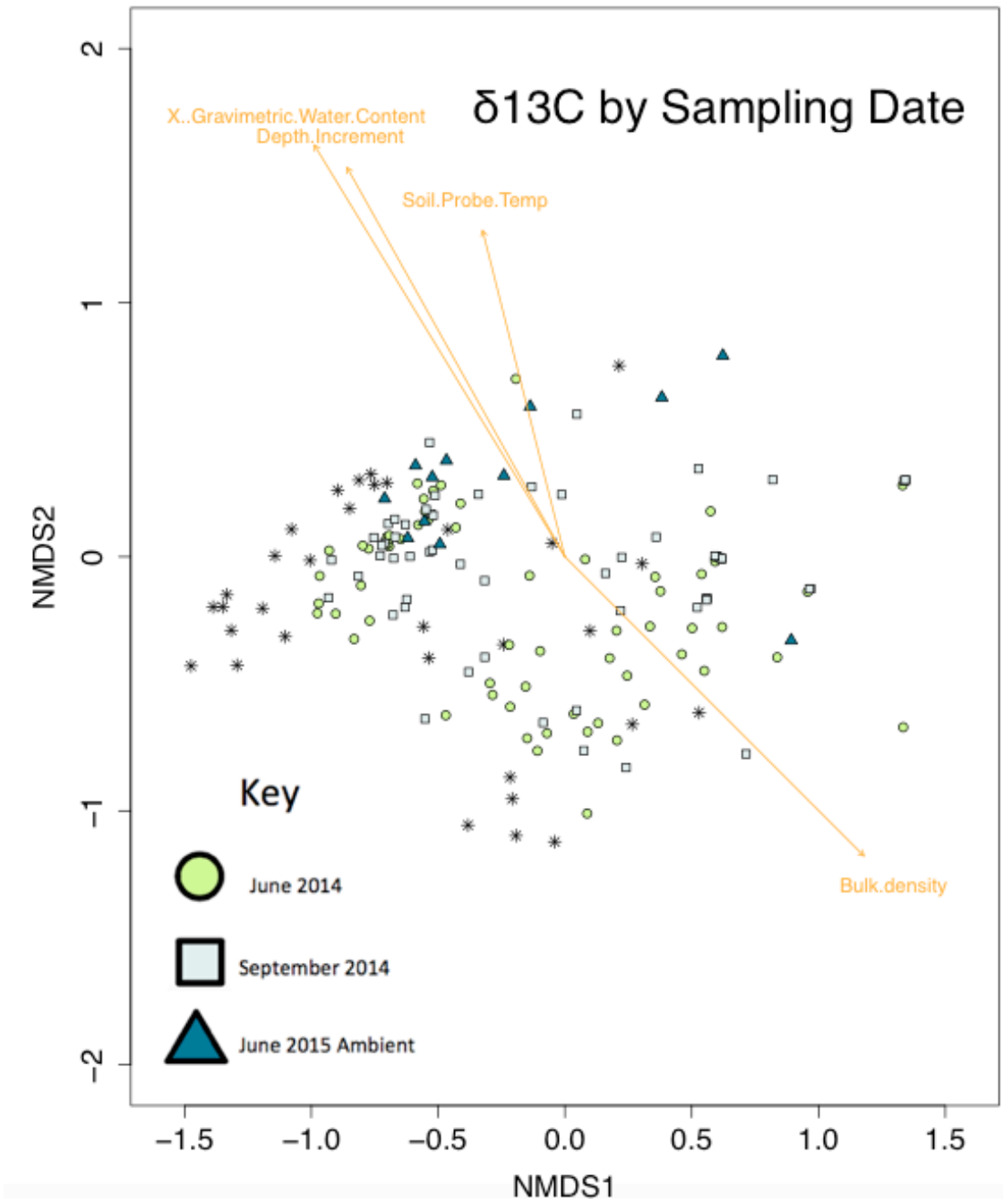

Figure 22. NMDS for δ13C by sampling date, P<.0001 PERMANOVA analysis. The final stress value after 20 runs for this ordination is .136.

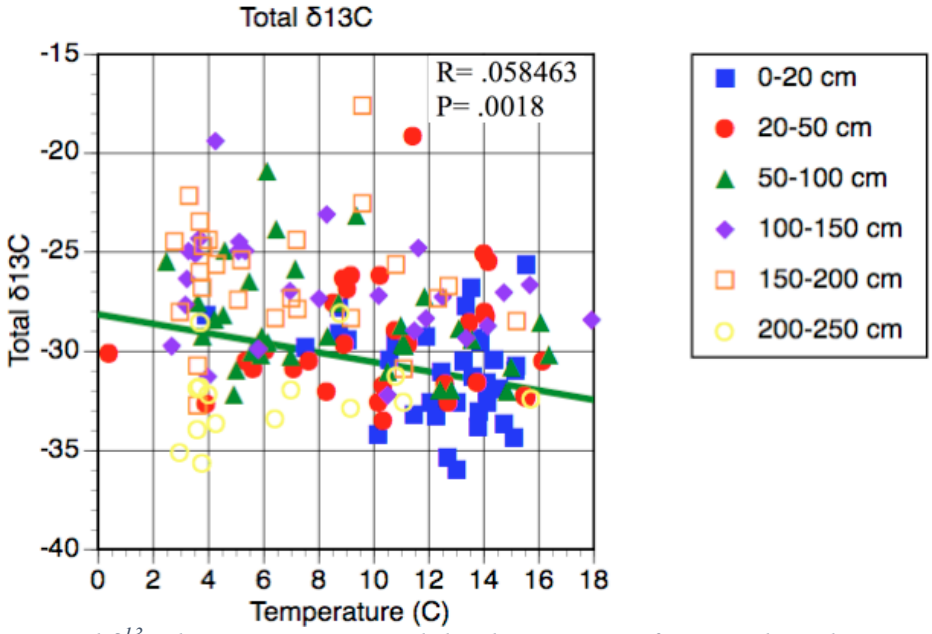

Figure 23. Total $\delta^{13}C$ by temperature and depth increment for sampling dates June 2014, September 2014, and June 2015 Ambient plots. Each plot is shown and colored by depth increment across the sampling dates June 2014, September 2014, and June 2015 ambient plots. A negative relationship was significant in the 50-100cm depth increment.

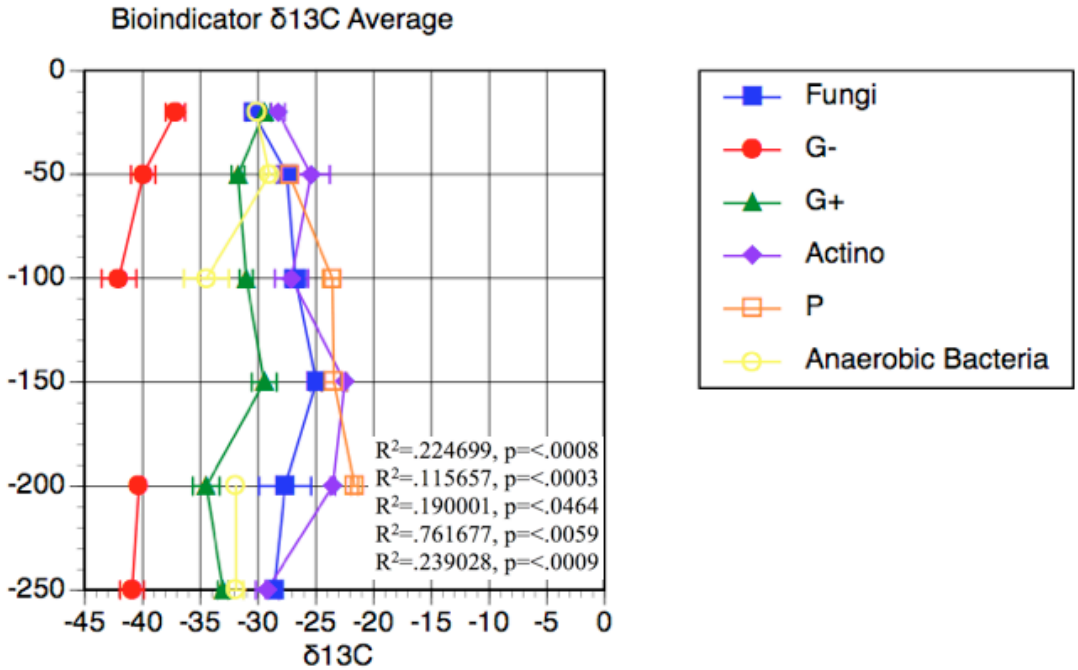

Figure 24. Bioindicator average $\delta^{13}C$ signatures by depth for sampling dates June 2014, September 2014, and June 2015 Ambient. Average bioindicator $\delta^{13}C$ signatures by depth is shown across 12 full plots, 2 partial, with 6 depth increments for 3 sampling dates. Standard error bars are shown.

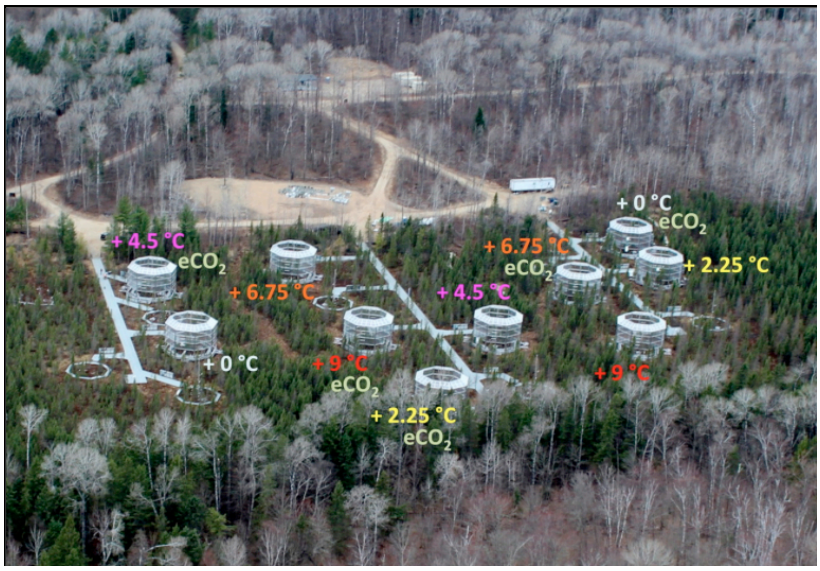

Figure 25. SPRUCE Plots Map by Treatment.

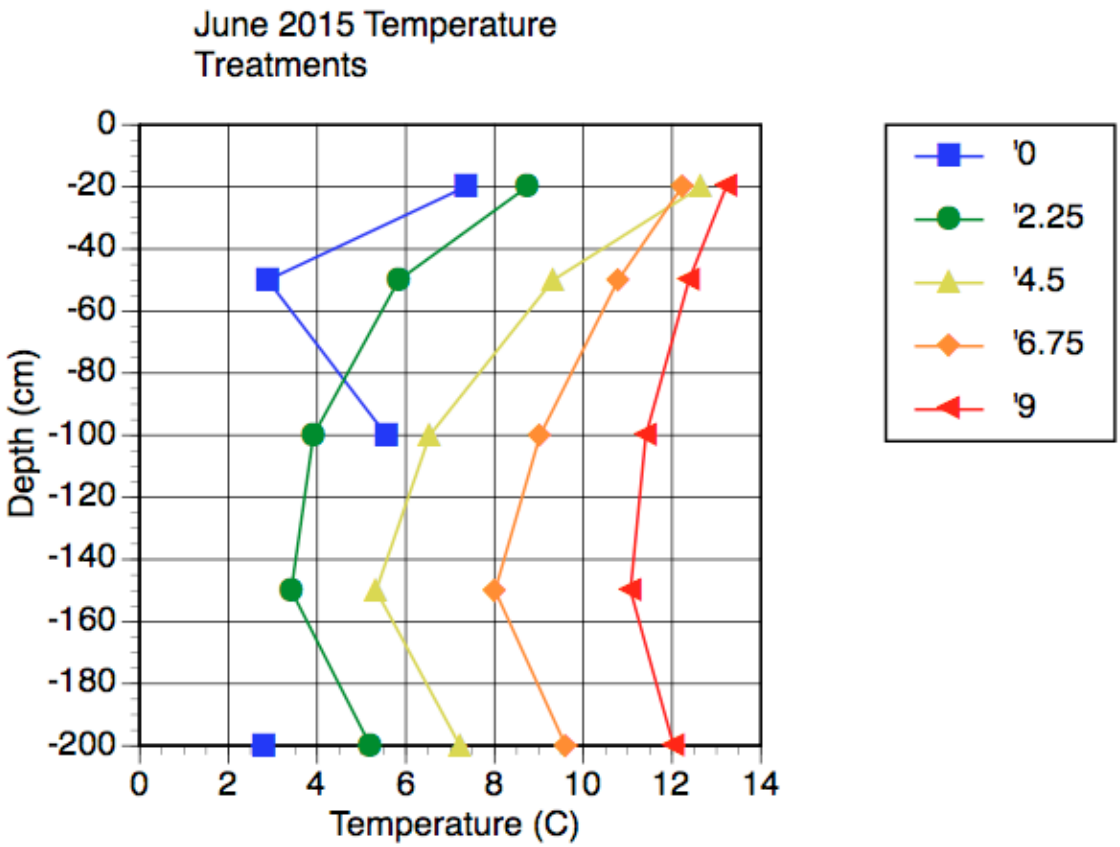

Figure 26. June 2015 Temperature treatments: Actual temperature by treatment averaged by treatment and shown by depth. Each treatment had two replicate plots.

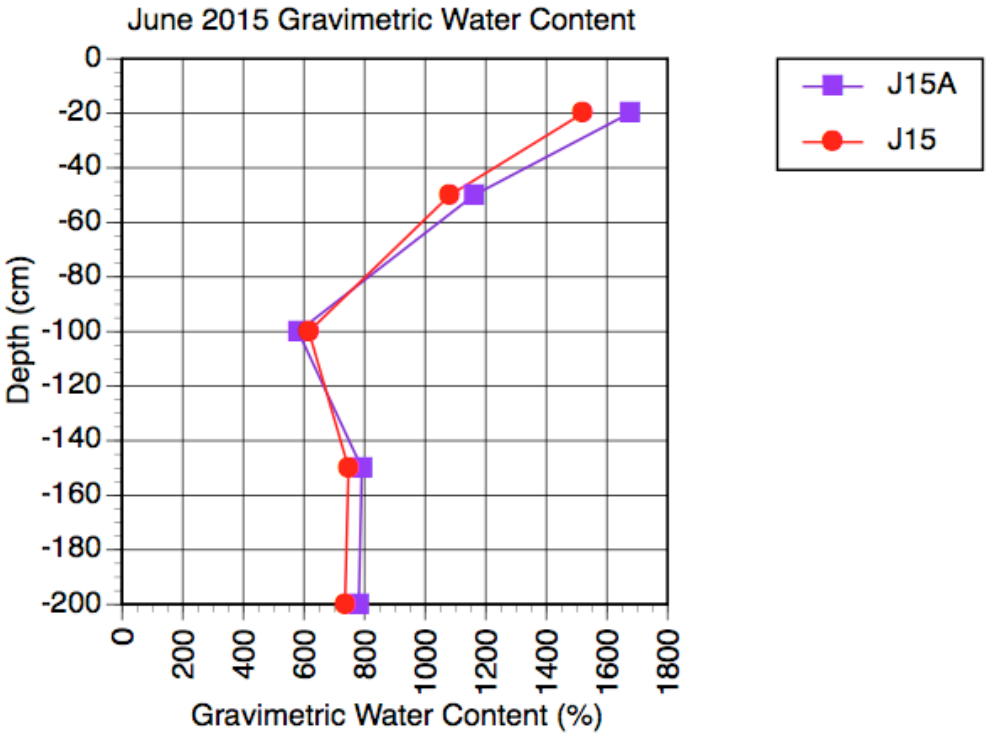

Figure 27. June 2015 treated vs ambient water content by depth. The difference was insignificant, but conditions were slightly more oxic in the treated plots within the surface meter of peat.

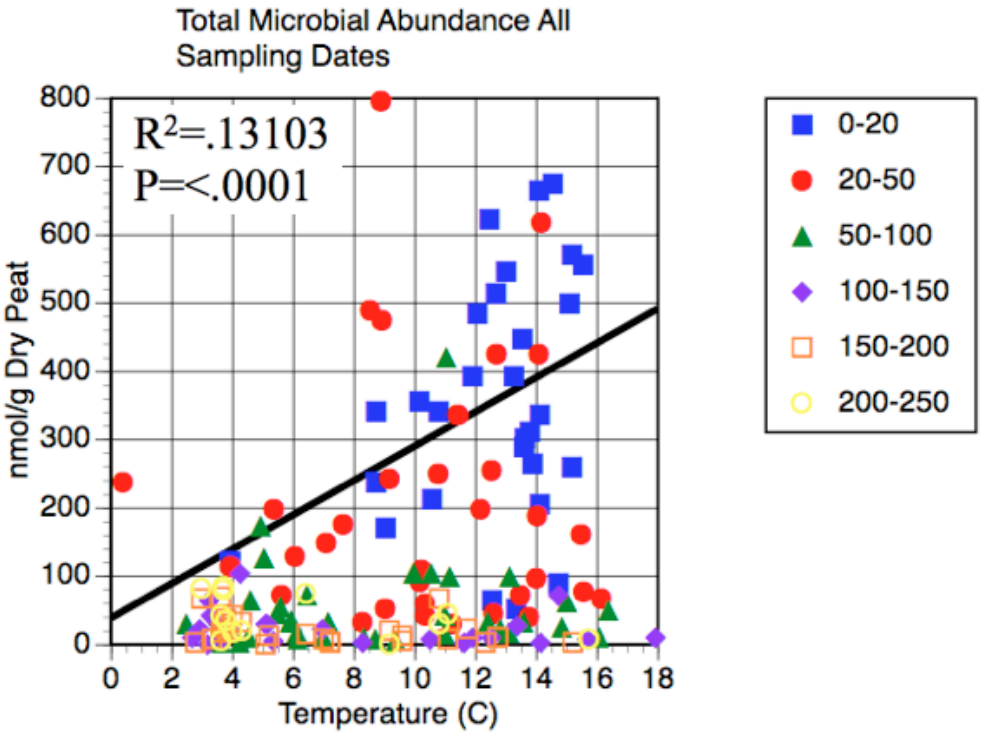

Figure 28. Total microbial abundance by temperature for all sampling dates showing a significant positive relationship. All 12 full and 2 partial plots plots in all 3 sampling dates are shown and colored by depth increment.

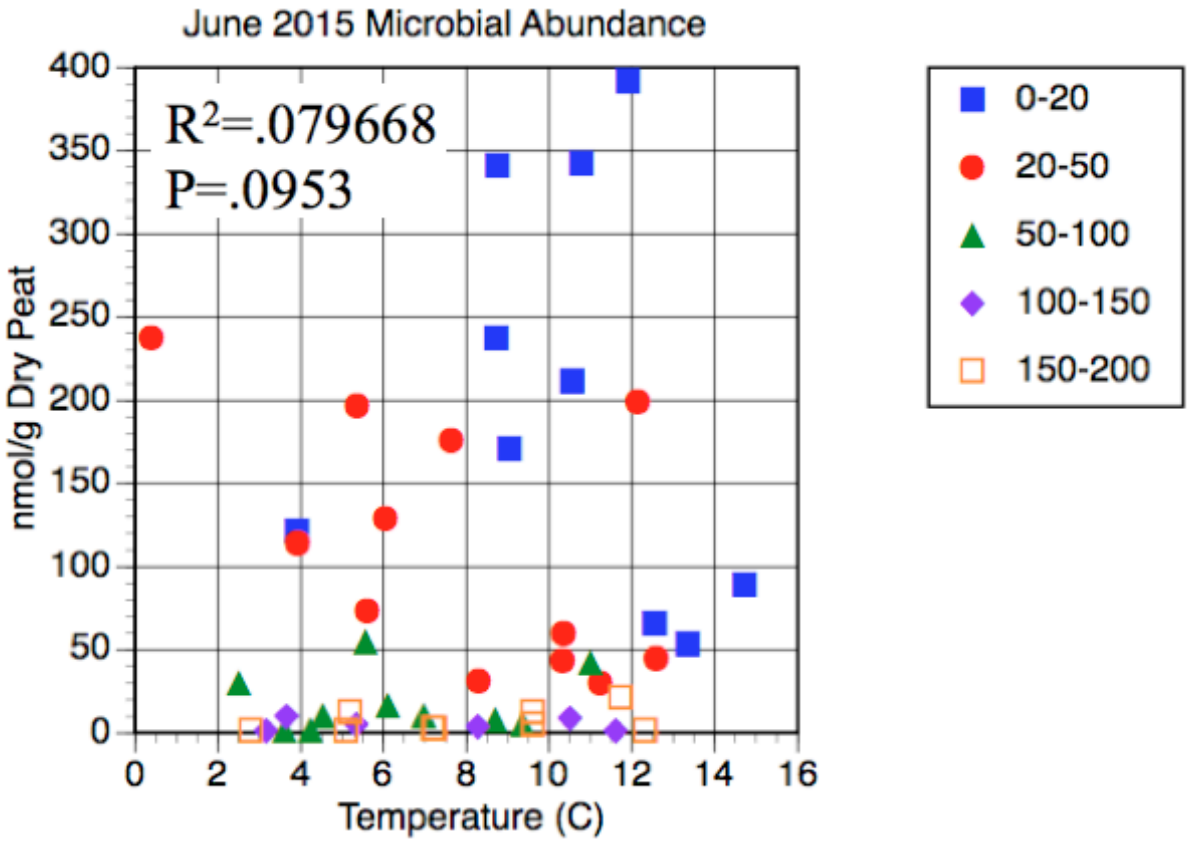

Figure 29. Total microbial abundance by temperature for June 2015 showing a statistically insignificant relationship, but slight vertical stratification of depth increments. All 12 full and 2 partial plots in the June 2015 sampling date are shown and colored by depth increment.

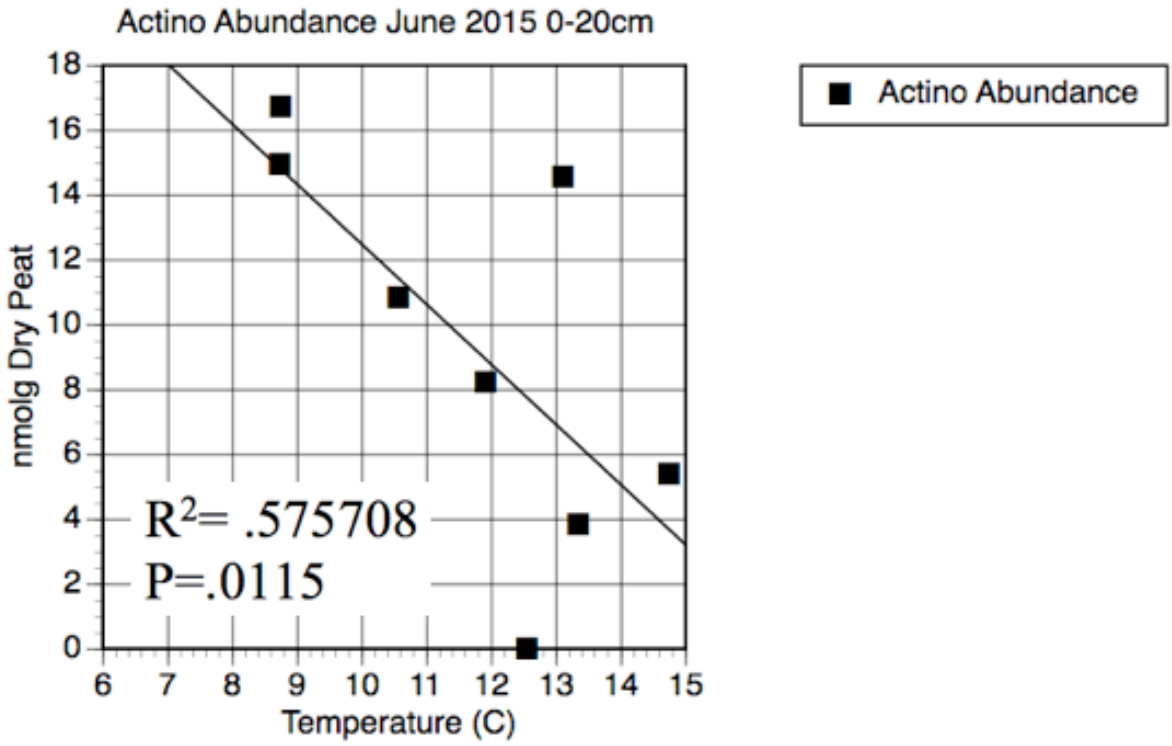

Figure 30. Actino Abundance by Temperature in June 2015 showing a significant negative relationship in the 0-20cm depth increment.

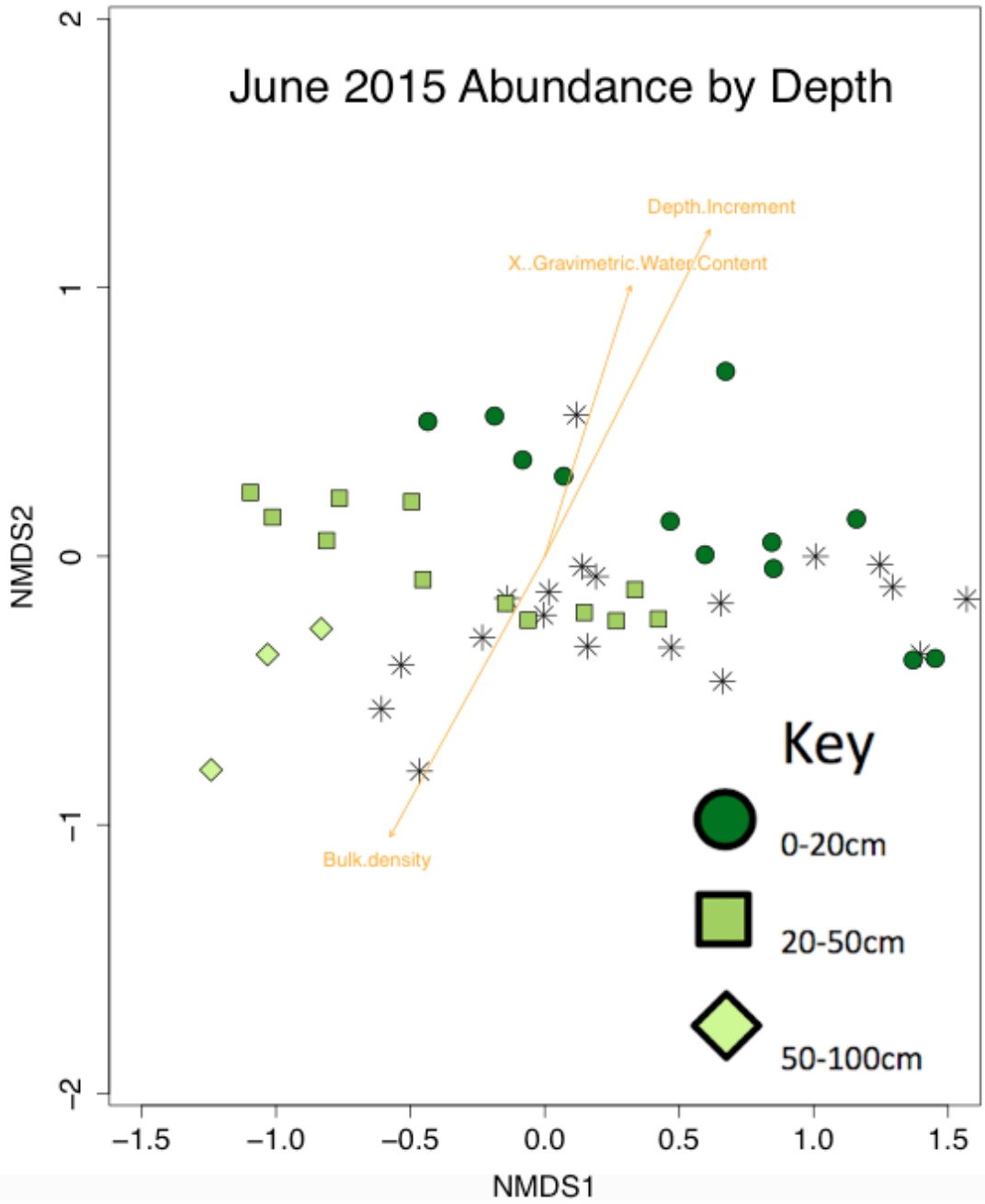

Figure 31. NMDS of Microbial Abundance by Depth in June 2015, P<.0001, PERMANOVA analysis. The final stress value after 20 runs for this ordination was .405.

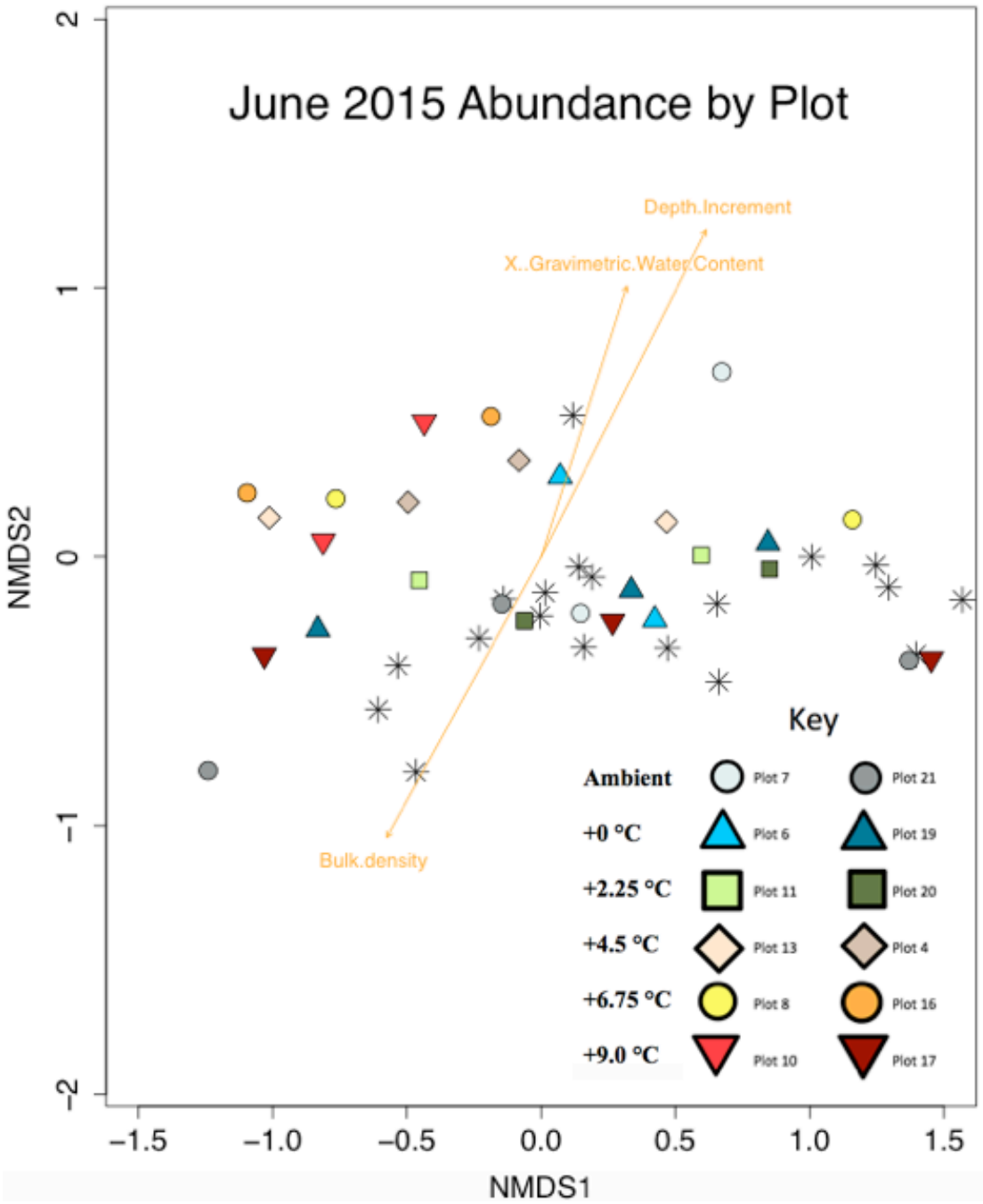

Figure 32. NMDS of Microbial Abundance by Plot, colored and shaped by treatment, in June 2015, P=456543, PERMANOVA analysis. The final stress value after 20 runs for this ordination was .405.

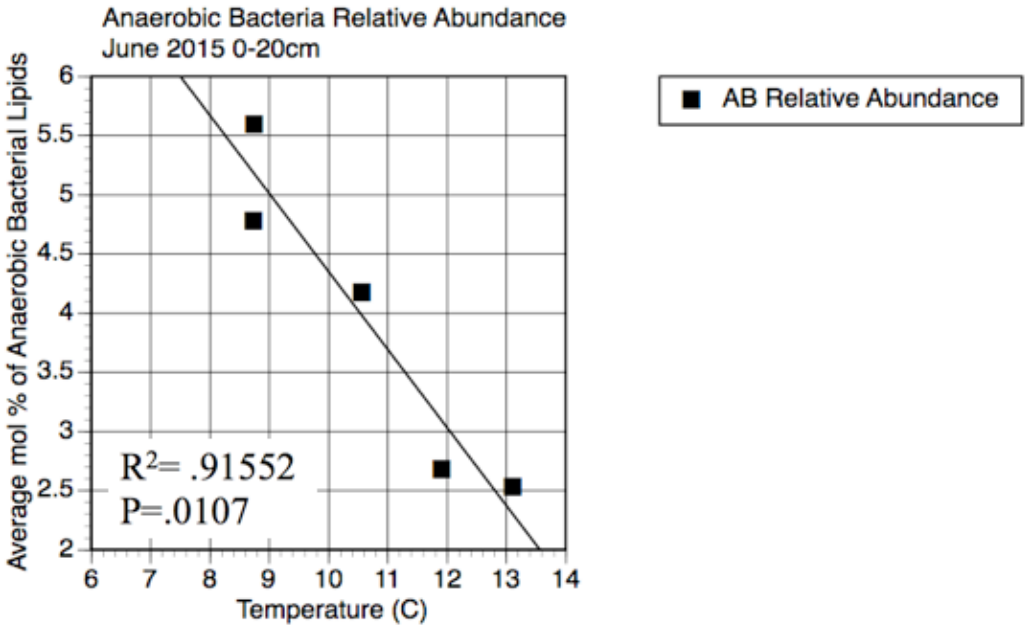

Figure 33. Anaerobic Bacteria relative abundance by temperature in June 2015 in the 0-20cm depth increment showing a significant negative relationship.

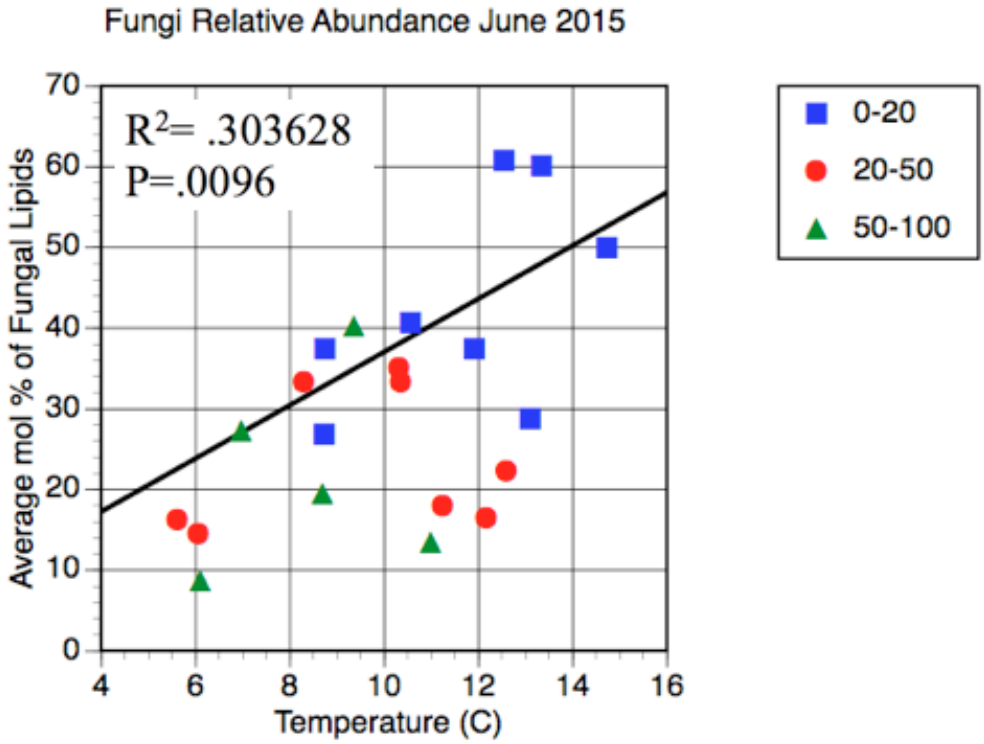

Figure 34. Fungi relative abundance by temperature for June 2015 showing a significant positive relationship. All 12 full plots are 2 partial plots for the June 2015 sampling date are shown and colored by depth increment.

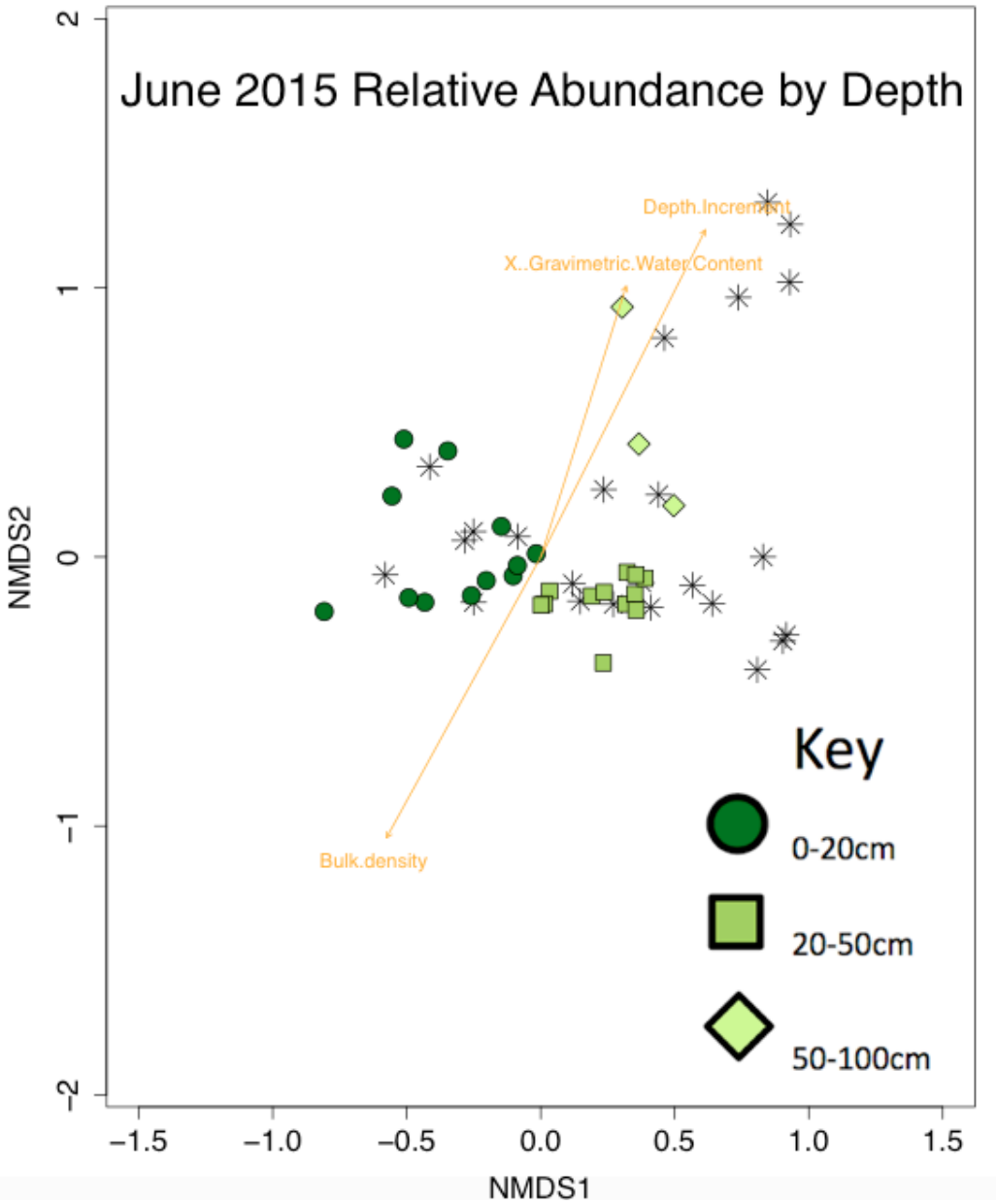

Figure 35. NMDS of Microbial Abundance by Depth in June 2015, P<.0001, PERMANOVA analysis. The final stress value after 20 runs for this ordination was .087.

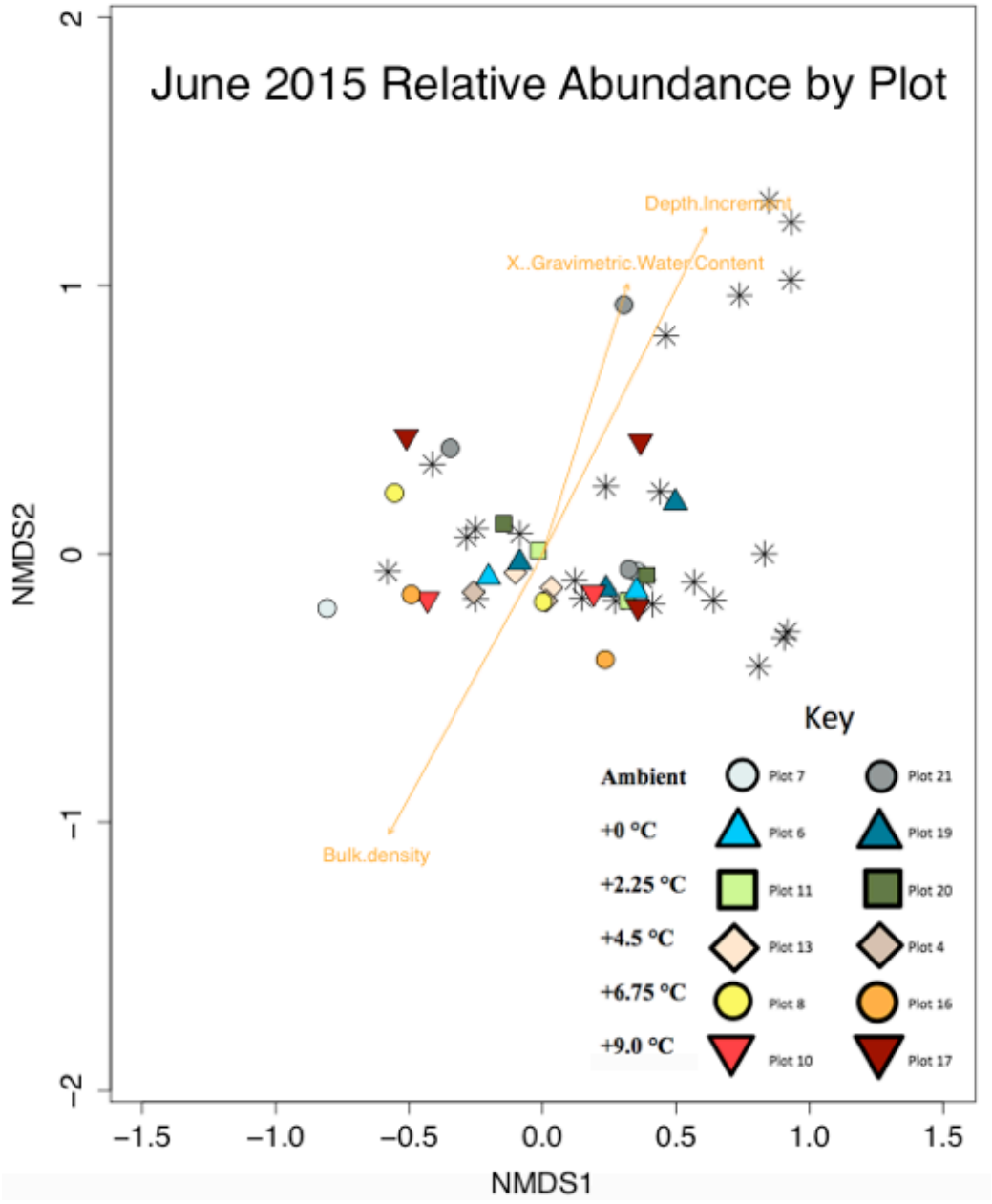

Figure 36. NMDS of Microbial Relative Abundance, colored and shaped by treatment and plot, in June 2015, P=456543, PERMANOVA analysis. The final stress value after 20 runs for this ordination was .087.

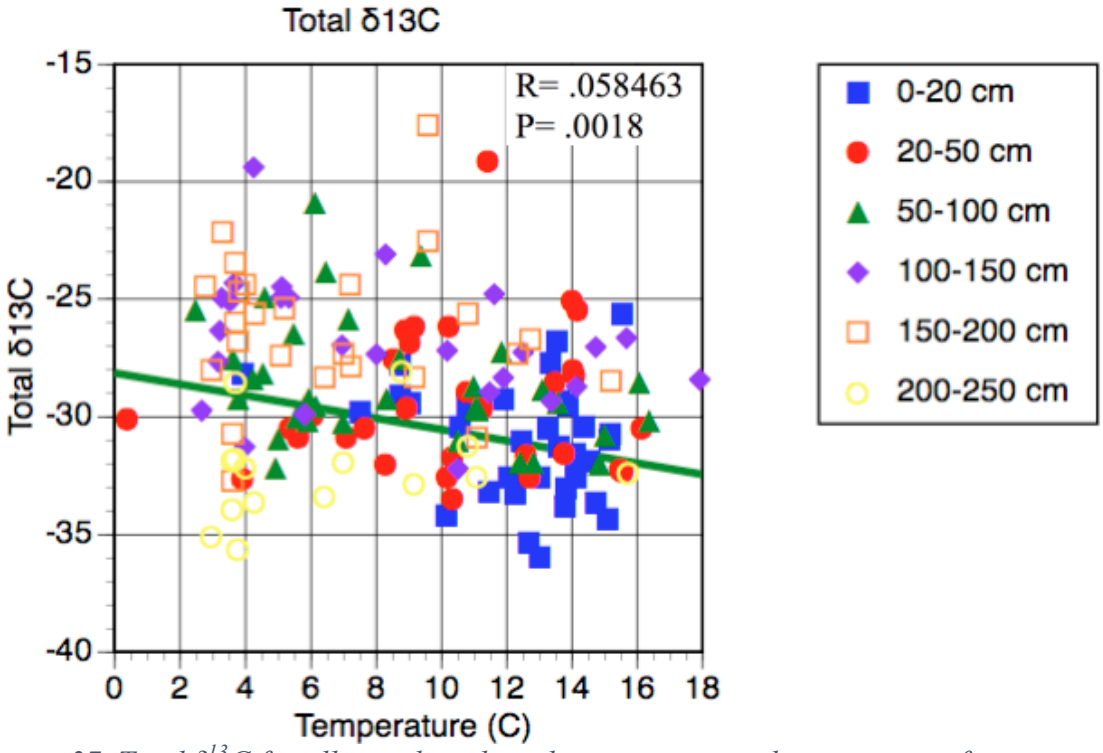

Figure 37. Total $\delta^{13}C$ for all sampling dates by temperature, showing a significant negative relationship. Total $\delta^{13}C$ by temperature and depth increment for sampling dates June 2014, September 2014, and June 2015 Ambient plots. Each plot is shown and colored by depth increment across the sampling dates June 2014, September 2014, and June 2015 ambient plots. A negative relationship was significant in the 50-100cm depth increment.

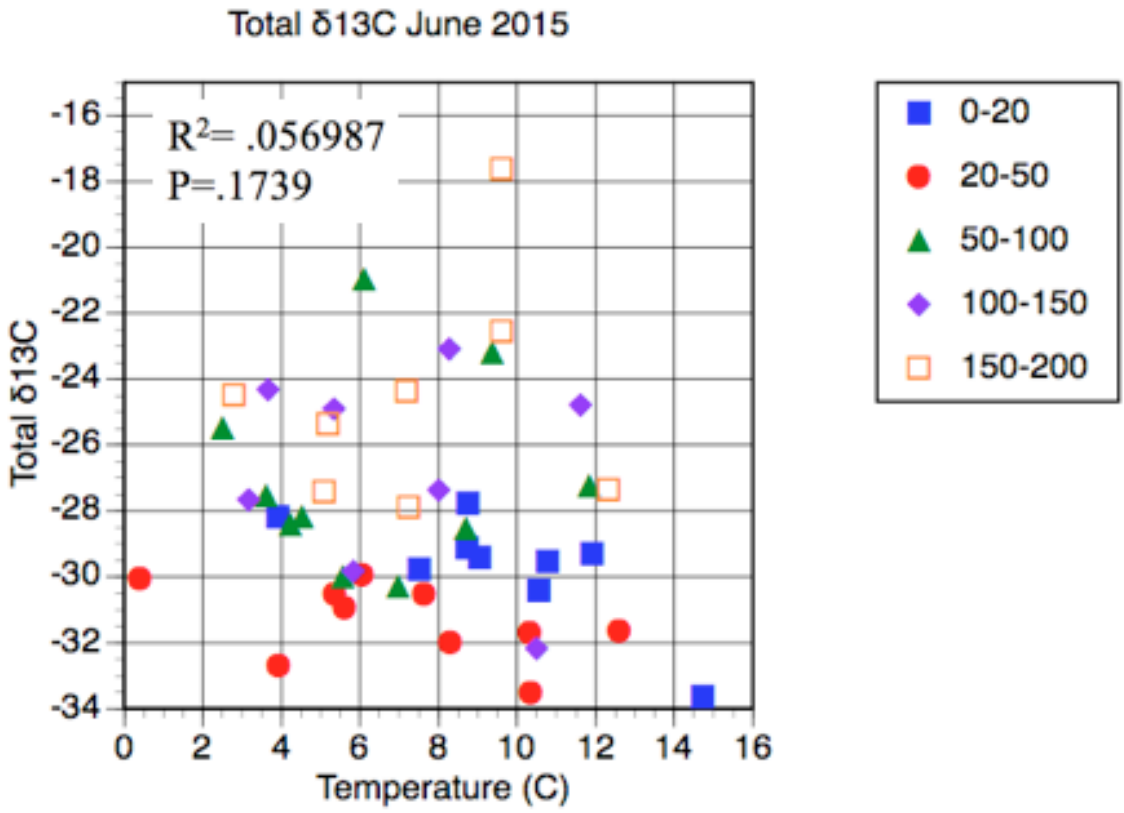

Figure 38. Total $\delta^{13}C$ for June 2015 by temperature, showing a lack of significant relationship. All 12 full plots are 2 partial plots for the June 2015 sampling date are shown and colored by depth increment.

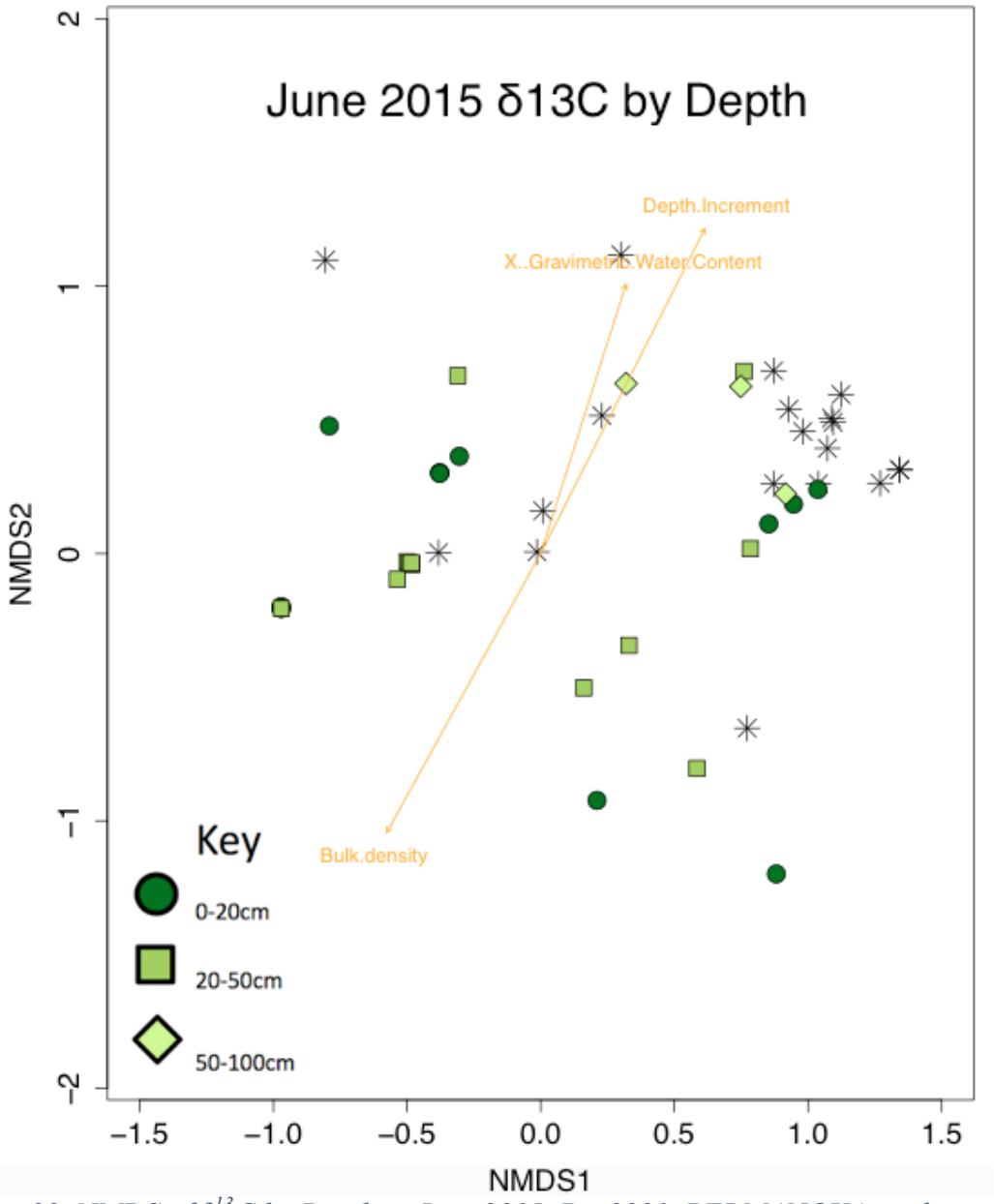

Figure 39. NMDS of $\delta^{13}C$ by Depth in June 2015, P<.0001, PERMANOVA analysis. The final stress value after 20 runs for this ordination was .797203.

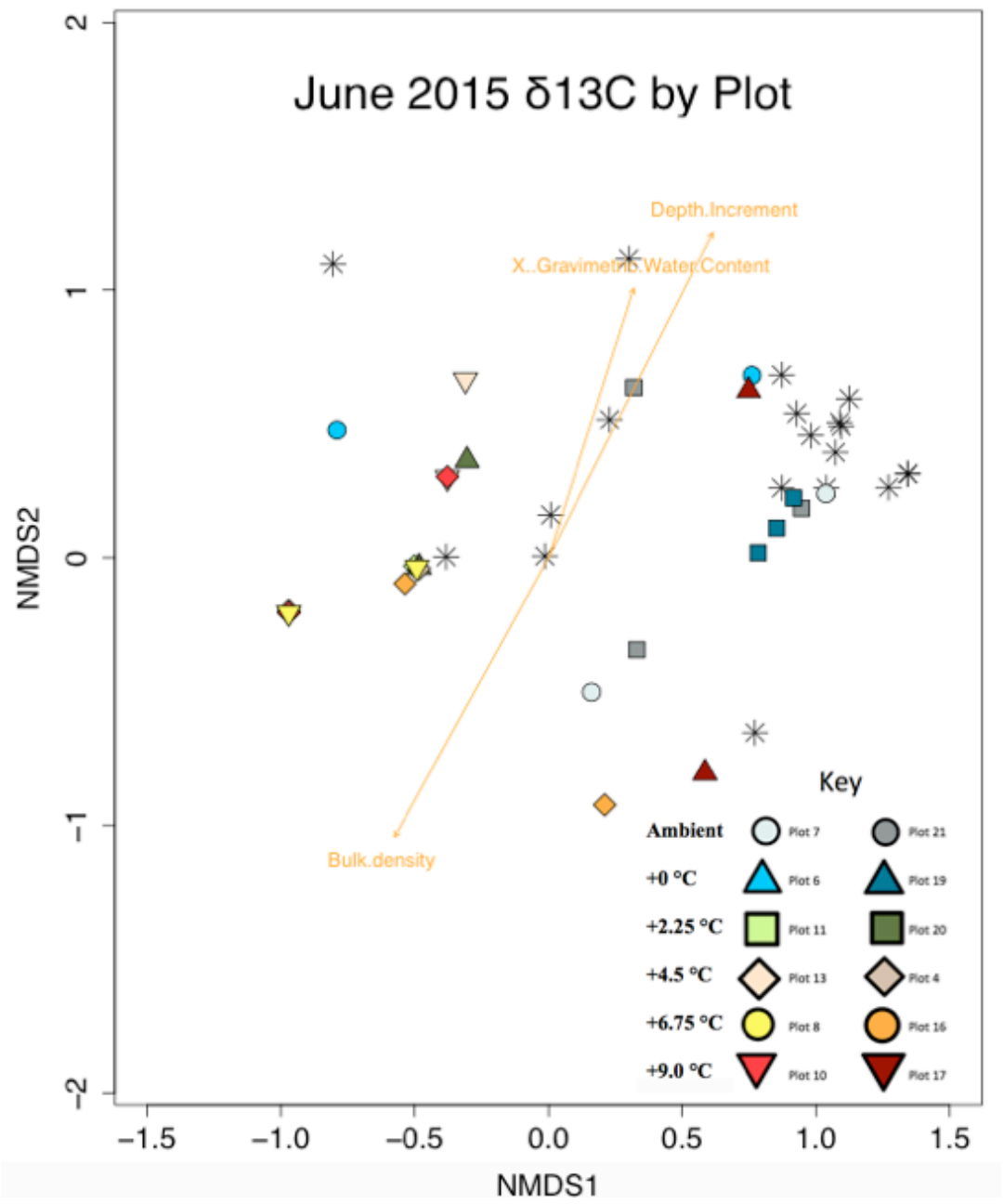

Figure 40. NMDS of $\delta^{13}C$ by by Plot, colored and shaped by treatment, in June 2015, P=456543, PERMANOVA analysis. The final stress value after 20 runs for this ordination was .091.

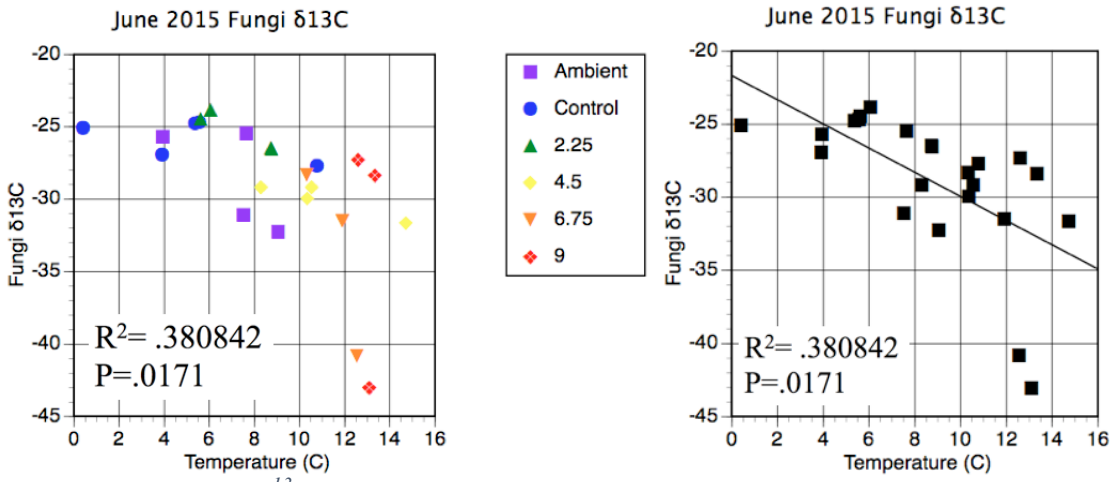

*Figure 41. **A** Fungi $\delta^{13}C$ by temperature, colored by temperature treatment, for June 2015 showing a significant negative relationship. **B** Fungi $\delta^{13}C$ by temperature for June 2015 showing a significant negative relationship.*

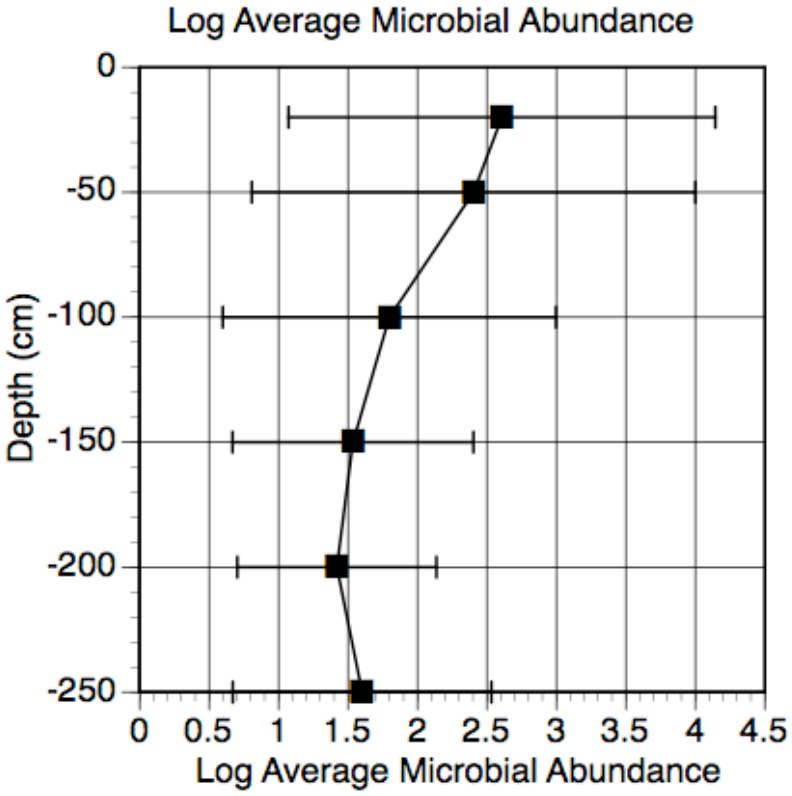

Figure 42. Log of total microbial abundance averaged across depth for the June 2014, September 2014, and June 2015 Ambient plots. Average microbial abundance by depth is shown across 12 full plots, 2 partial, with 6 depth increments, and across 3 sampling dates of June 2014, September 2014, and the June 2015 ambient plots. Standard error bars are shown.

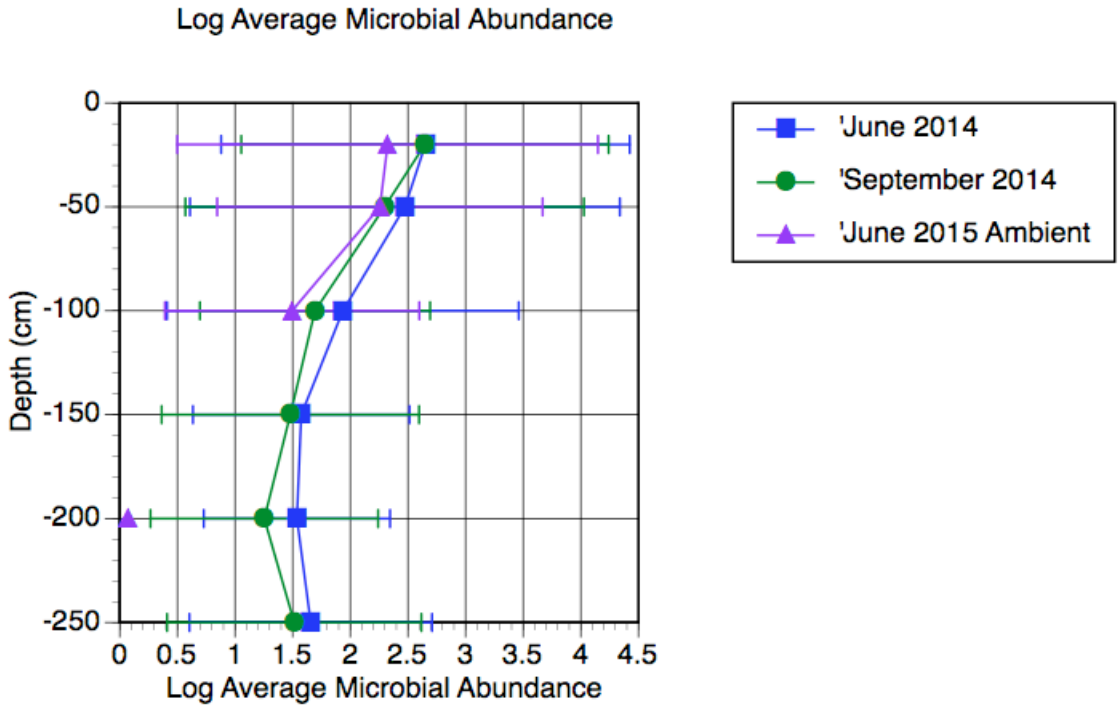

Figure 43. Log of average microbial abundance across depth by sample date. Average microbial abundance by depth is shown across 12 full plots, 2 partial, with 6 depth increments for 3 sampling dates. Standard error bars are shown.

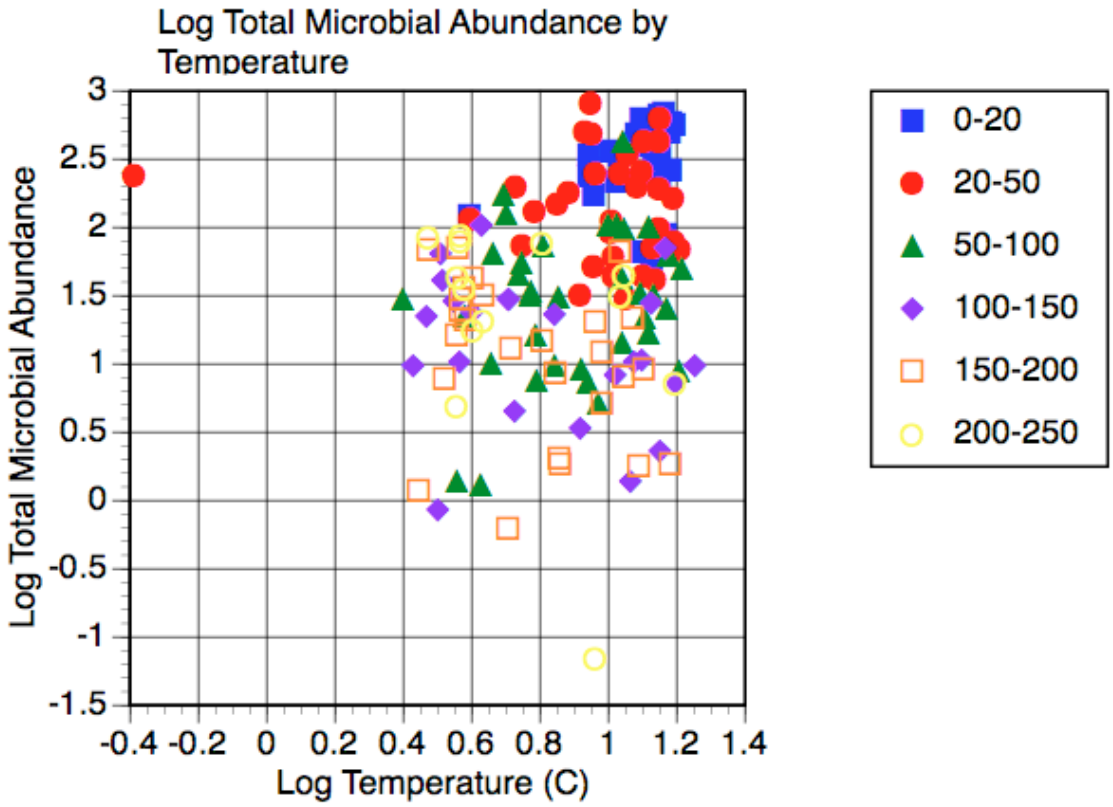

Figure 44. Log of total microbial abundance by temperature, showing a significant positive relationship. Total microbial abundance is shown and colored by depth increment. Each plot is shown and colored by depth increment across the sampling dates June 2014, September 2014, and June 2015 ambient plots.